

COLLOID SYMPOSIUM MONOGRAPH

PAPERS PRESENTED AT THE THIRD NATIONAL
SYMPOSIUM ON COLLOID CHEMISTRY
THE UNIVERSITY OF MINNESOTA, JUNE, 1925

EDITED BY

HARRY N. HOLMES

CHAIRMAN, COMMITTEE ON THE CHEMISTRY OF COLLOIDS
NATIONAL RESEARCH COUNCIL

ASSISTED BY

HARRY B. WEISER, *Chairman-Elect*

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FOREWORD

With this Monograph the Colloid Committee of the National Research Council firmly establishes a series of important annual publications. In Volume I were collected the papers presented at the First Colloid Symposium (University of Wisconsin); in Volume II the papers read at the Second Colloid Symposium (Northwestern University) were published; and in Volume III we now offer the papers read at the Third Colloid Symposium (University of Minnesota).

As usual, theory and application are both given adequate attention. Medicine and agriculture are prominent, but so are orientation of molecules, surface tension, and electrokinetic potential.

Professor Herbert Freundlich, as the foreign guest of honor at the Symposium, made a notable contribution to the discussions. His paper is a valued part of this volume.

HARRY N. HOLMES.

Oberlin College,
Oberlin, Ohio.

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COLLOID SYMPOSIUM MONOGRAPH

ON THE ELECTROKINETIC POTENTIAL.

BY HERBERT FREUNDLICH

The electrical phenomena with which we have to deal in colloidal chemistry are of very different kind from those which are well known in electrochemistry. In the latter domain of science we have the electromotive force of voltaic cells, we have electrolysis too, and Nernst's thermodynamic theory of the electrode potential, and the theory of diffusion and of reaction-velocity go pretty far in explaining the main facts. In colloidal chemistry we have come across certain electrical phenomena, which had been neglected by electrochemistry. These are the so-called electrokinetic phenomena, electrosmosis, cataphoresis, stream-potential, the potential of moving particles and some others. They have been studied in so many respects during the last years, that I only need call the chief facts back to your mind.

In electrosmosis and cataphoresis an electromotive force acts on an interface, the electric current flowing tangentially to the plane of it, and causing a movement of one phase against the other. For instance, in electrosmosis a fine capillary lies between the electrodes and the electromotive force causes a movement of the fluid through the capillary; in cataphoresis on the other hand, the particle of a different phase, it may be solid, fluid or gaseous, is moved through a liquid under the influence on the electric current. In stream-potential and the potential of moving particles, the relationship is reversed: the movement of one phase against the other causes an electromotive force. In stream-potential a fluid is pressed through a capillary lying between two electrodes and the movement of the fluid causes an electric current to flow from one electrode to the other; in the potential of moving particle, the particles fall for instance in a column of liquid and set up an electric current between two electrodes, one at the upper, one at the lower end of the column. That these four phenomena are so closely related is guaranteed by very trustworthy experiments.

Now the theory of these electrokinetic phenomena was given by

Helmholtz¹ about 50 years ago, and it covered the purely physical facts so well, that not until two years ago, certain corrections by Debye and Hückel² were really found to be necessary, and even these are not very trenchant. Helmholtz assumed a double layer on the interface between the two phases, and the potential of this double layer—we may call it ζ , in contrast to the ϵ -potential of Nernst—is a characteristic quantity entering into all his formulæ. These are the following: for electrosmosis we have

$$v = \frac{r^2 \zeta H D}{4\eta}$$

Here v is the amount of fluid flowing per second through a capillary with a radius r under the influence of an outer potential-gradient H ; D is the dielectric constant of the fluid, generally the dielectric constant of water is used, η its constant of viscosity. I must add that Helmholtz did not take account of the dielectric constant, a point which I must consider more fully later on. For cataphoresis Helmholtz wrote

$$u = \frac{\zeta H D}{4\pi\eta}$$

Here u is the cataphoretic velocity of the particle which Helmholtz believed to be independent of its shape. Debye and Hückel showed that this is not the case, but that the numerical quantity in this formula changes with the shape of the particle. For a spherical particle they found

$$u = \frac{\zeta H D}{6\pi\eta}$$

For the stream-potential we have

$$S = \frac{\zeta P D}{4\pi\eta\lambda}$$

Here S is the potential between the two electrodes caused by the movement of the liquid, P is the pressure with which the liquid is forced through the capillary, λ the electrical conductivity of the liquid.

Helmholtz did not discuss the nature of this electrokinetic potential, how is it changed by electrolytes or non-electrolytes. He believed it to be caused by a double-layer in the strict sense of the word: Two electrically charged layers lying opposite to one another in a distance of the diameter of one molecule. He therefore also neglected the influence of the dielectric constant.

The rapid development of electrochemistry since 1887 naturally leads to the question: What relationship is there between this electro-

kinetic potential ζ and the thermodynamic potential of Nernst ε , so useful in the theory of voltaic cells? But first the reawakening of colloid chemistry in the years around 1900 called forth the first attempt of answering it. Billiter³ tried to explain the electrokinetic phenomena by simply assuming that the electrokinetic potential is identical with the thermodynamic potential. The extraordinary results he was led to, made this assumption very doubtful. But one idea introduced by Billiter proved to be valuable and was retained since. He did not consider the double-layer to be one in the old strict sense of the word, but believed it to be "dissociated," a part of the ions in the layer lying on the liquid side having diffused into the liquid. The charge of the layer therefore does not lie in a plane but in a certain space extending sensibly into the interior of the liquid. It seems to be well established that heat motion must necessarily cause such a dissociation or diffusion of the double layer. As a consequence of this view Pellat⁴ introduced the dielectric constant into Helmholtz formulæ.

To explain the relationship between the electrokinetic potential and the thermodynamic potential, it would be the simplest and most direct way to measure them both for the same interface. This cannot be done so very easily. The ε -potential is best known for metal electrodes; but it is not so simple to determine the ζ -potential for metals. Cataphoretic experiments with metal-sols are feasible, but it seems rather doubtful, if the metal surface of colloidal particles may be regarded as identical with the surface of metal-electrodes. Rona and I⁵ preferred to try glass surfaces for comparing the two potentials. Electrosmosis and especially stream-potentials may be measured exactly in glass capillaries and they give the value of the electrokinetic potential. The fluid is pressed through a glass capillary lying between two electrodes and the potential difference between these two electrodes is measured using a bridge and a suitable electrometer. Not so simple is the measurement of an ε -potential of glass-electrodes. But this also is possible according to the experiments of Haber and Klemensiewicz.⁶ They dipped a very thin glass bulb, having a wall less than 0.1 mm. thick, into a beaker containing a solution of an electrolyte. The bulb also contained an electrolyte-solution, which remained the same in all experiments, and a platinum electrode. A normal-electrode immersed into the beaker. The potential difference between the electrodes was measured with an electrometer. If the nature and concentration of the electrolytes in the beaker were changed, the potential difference between the glass bulb-electrode and the normal-electrode change in a regular way. Haber and Klemensiewicz found, that the glass bulb behaved very like an H-electrode of constant concentration. New measurements by Horovitz⁷ have shown that the glass electrode is not only sensible to the change of concentration of the H- and OH-ion,

but also to the change of some other ions contained in the glass, especially the alkali cations. In any case the potential of the glass bulb behaved like a thermodynamic potential ε ; it may be compared to the potential of an alloy.

In this way it was possible to compare the ζ -potential of a glass-surface with its ε -potential. If they were identical or very closely connected they ought to be influenced by different electrolytes in a similar manner; if this was not the case, they had to be considered as being to a high degree independent from one another. The experi-

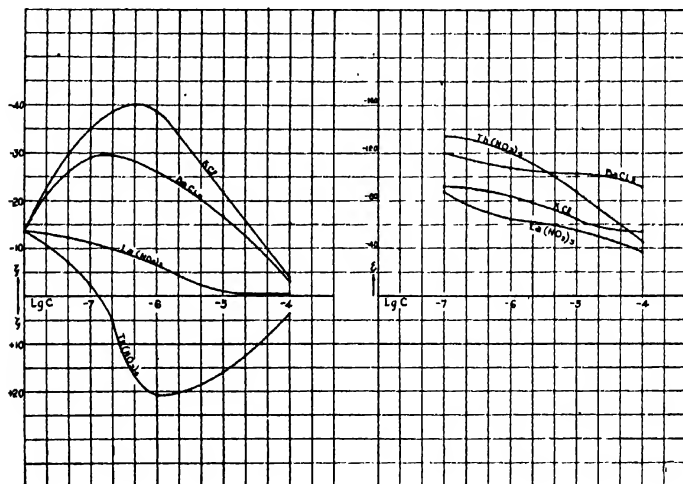


FIG. 1.

ments showed that the latter proved to be true. The outstanding influence of the H-ion so characteristic for the potential of the glass-bulb-electrode was not specially marked for the ζ -potential. On the other hand, capillary-active substances, dyestuffs and others, and ions of high valency change the ζ -potential strongly, but have no marked effect on the potential-difference of the glass-electrode. This resulted already from the experiments of Rona and myself and were confirmed by new and more copious measurements of Ettisch.⁸ He compared for instance the influence of salts with cations of different valencies. The two diagrams (Figs. 1 and 2) show how totally different the curves of the ζ - and ε -potential behave. The absciss is the logarithm of the concentration of salt, the ordinate the ζ - and ε -potential. I especially wish to draw your attention to the behavior of Th(NO₃)₄. It changes the

sign of the ζ -potential in very low concentration (about 10^{-7} mol), whereas the sign of the ε -potential is not changed in a concentration 1000 times as high. A similar row of experiments were executed with salts of some derivatives of quinine, with the chlorides of optochin, eucupin and vucin, three substances with strongly rising capillary activity. The difference in the behavior of the ζ -c- and ε -c-curves is again very marked, the strongly capillary-active vucin being able to change the sign of the glass for the ζ -potential, whereas nothing similar was observed for the ε -potential.

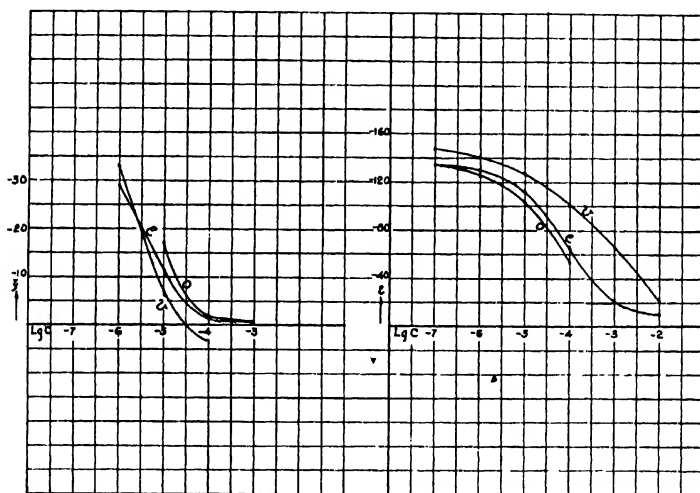


FIG. 2.

There seems to me to be no doubt that the two potentials are widely independent from one another. How is this to be explained? One main point is, that we are not measuring the same quantity. If we measure the thermodynamic potential ε in voltaic cells we measure *vertically* to the interface between two phases, the electrokinetic potential ζ on the other hand in electrokinetic experiments is measured *tangentially* to the interface between two phases. In the first case, we determine the potential-difference between the surface layer of the solid electrode itself and the interior of the liquid, in the case of electrokinetic experiments, we have to assume that a layer of liquid firmly adheres to the solid surface, and on measuring the ζ -potential we determine the potential-difference between this firmly adhering layer of liquid and the interior of the liquid. There is a movement

between two liquid layers, not between the liquid and the solid wall itself. This assumption would be untenable if he had a double-layer in the strict sense of the word, as Helmholtz introduced it, because there would be no change of potential between the two liquid layers. But if we assume a dissociated or diffused double-layer, the electric charge of the one layer not lying in a plane, but in a certain space extending sensibly into the interior of the liquid, then there is no difficulty in assuming also a potential difference between the adhering layer of liquid and the interior of the liquid. The following diagram (Fig. 3)

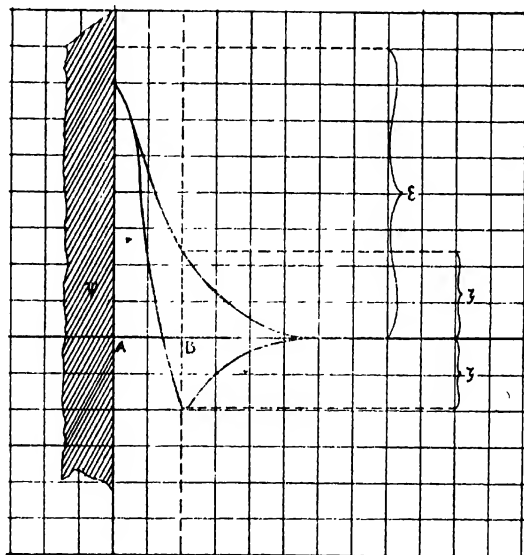


FIG. 3.

gives a rough picture of this view, the abscissa being the distance from the solid wall, the ordinate the potential. To the left of *A*, we have the solid wall or the electrode, between *A* and *B* the layer of liquid adhering to the wall, to the right of *B*, the movable interior of the liquid. The curves 1 and 2 give two possible slopes of the potential-curves and show how the ϵ -potential may be the same, whereas the electrokinetic potential ζ is not only smaller but even different in sign. The ϵ -potential is mainly influenced by those ions which really enter the solid electrode; if we have a metal electrode, the ions of the metal will alone decide its value; in the case of the glass-electrode H-ion and the ions of the alkali metals are specially able to enter the surface

of the glass itself. The ζ -potential on the other hand is dependent on all ions accumulated in the surface layer, that is to say, on all ions (and other substances) adsorbed in the interface. That is the reason why capillary-active substances so strongly influence the electrokinetic potential.

O. Stern⁹ has discussed this train of thought in a more exact and detailed form. Most physicists have scruples in assuming an adsorption or adhering layer thicker than one layer of molecules. I personally cannot quite share their doubts, although I do not deny that in

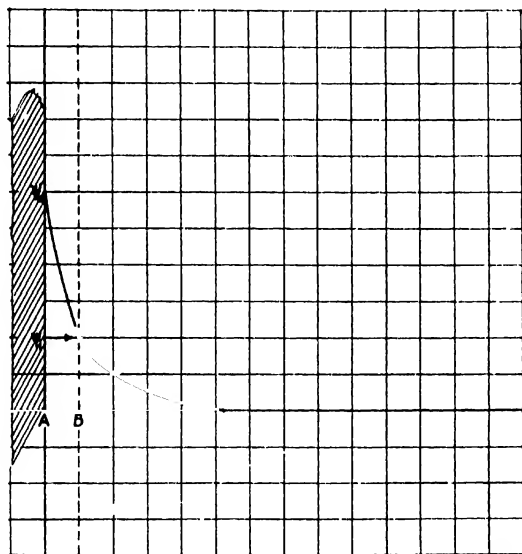


FIG. 4.

many cases the adsorption layer really seems to consist of only one layer of molecules. Other facts indicate that the double layer is not dissociated strongly, the majority of ions being bound to a double layer similar to that assumed by Helmholtz, only a comparatively small number of ions having diffused into the interior of the liquid. This amount and their position in the neighborhood of the interface again depends mainly upon their adsorption. It is the specific adsorption of each single ion which has to be introduced. The two diagrams (Figs. 4 and 5) give an idea of Stern's views. In the first one we have the case that the adsorbability of the two ions is not very different. ψ_0 is Nernst's potential, ψ_1 the potential between the layer adhering to the

solid and the interior of the liquid. Stern assumes that this layer is only one molecule thick; he therefore identifies ψ_1 with the ζ -potential. If the adsorbability of the ions is very different the second diagram comes in question. You see that ψ_0 is very different from ψ_1 , it may even have the opposite sign.

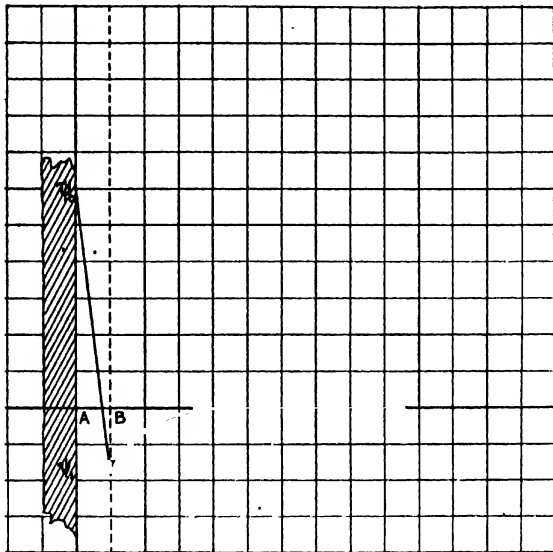


FIG. 5.

The formula Stern developed, neglecting many points which are perhaps not negligible, is

$$K_0(\psi_0 - \psi_1) = Fa_s \left[\frac{1}{1 + \frac{1}{c}} \frac{\Phi^- - F\psi_1}{RT} + \frac{1}{1 + \frac{1}{c}} \frac{\Phi^+ + F\psi_1}{RT} \right]$$

Here Φ^+ and Φ^- are the adsorption potentials, the amount of free energy necessary to transport one molecule of each ion out of the interior of the solution into the interface. Stern regards these quantities as constant. It may be preferable to consider them as a function of the concentration of the ions c and to write in their stead $f^+(c)$ and $f^-(c)$. K_0 is a constant, a_s the maximum amount of ions which may be adsorbed; F , R and T have their well-known meaning. In any case

the formula makes clear that ψ_1 —and ψ_1 is identical with ζ —is no simple function of c , and that therefore complicated curves with a maximum and minimum, like those shown in Figs. 1 and 2, are not surprising. We are not able to calculate the value of ψ_1 as a function of c , because we do not know anything about the specific adsorbability of the single ions, we do not know $f^*(c)$ and $f(c)$. He who first finds the way to measure them, will also be able to calculate the ζ - c -curves.

Till now I have only been treating the case of an interface between a solid and a liquid. But also when we have an interface between two liquids or between a liquid and a gas, the ζ -potential has to be distinguished from the ε -potential. In the case of the interface of two non-miscible fluids the measurements can be done in the following manner. For the ζ -potential, the cataphoresis of small droplets of the second liquid in the watery solution may be used; for the ε -potential, the electromotive force of voltaic cells has to be determined set up like those which Beutner¹⁰ and Baur¹¹ have examined.

normal-electrode	water solution 1	organic liquid	water solution 2	normal-electrode
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The one water solution is kept constant, the other is changed and in this way the relative influence of different electrolytes upon the potential of the interface water-organic liquid is measured. Here again the independence of the two potentials was in some cases striking.¹² For instance, with phenol as second fluid, AlCl_3 changed the sign of the electrokinetic potential, turning it from negative to positive—the influence of AlCl_3 was compared with that of KCl —; the thermodynamic potential on the other hand was only slightly more negative with AlCl_3 than with KCl , the electrode on the AlCl_3 side being positive against the electrode on the KCl side.

Frumkin¹³ has done remarkable measurements of the potential difference on the surface of watery solutions against the gas above them, determining the potential vertically to the plane of the interface. It would lead me too far, to discuss his methods of measurement. The potentials he found were absolutely dependent on the composition of the surface layer of molecules limiting the liquid phase. This layer is an adsorption-layer, the composition of which may be derived from the change of surface-tension under the influence of dissolved substances according to Gibbs' adsorption law.

Frumkin's experiments verified excellently everything that was to be expected as consequence of an adsorption on the basis of the changes of surface-tension. Since we have such a pronounced influence of adsorption here, the notion might be cherished that the potential be-

tween liquid and gas determined in this way might be identical with an electrokinetic potential on the interface liquid-gas. The latter has been measured by McTaggart,¹⁴ who determined the velocity of cataphoresis of small gas bubbles in water solutions. But this ζ -potential was not identical with the potential measured by Frumkin. In the experiments of McTaggart, the same influence of ions of high valency was found as is known for other cataphoretic experiments. Th-ion for instance changed the sign of the charge exactly as it did in the measurements of stream-potential, mentioned before. This influence of high valency was not found by Frumkin. We therefore may assume that also in the case of an interface liquid-gas a part of the electric charge is distributed into the interior of the liquid and causes a ζ -potential in electrokinetic experiments, but does not show up in experiments like those of Frumkin, which were executed vertically to the interface.

It therefore seems correct to assume a dissociated double-layer on every interface, in any case if a liquid phase is one of the components, and to distinguish systematically between the ζ - and the ϵ -potential.

Kaiser Wilhelm-Institut,
Berlin-Dahlem, Germany.

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MOLECULAR WEIGHT AND SOLUTION

BY WILDER D. BANCROFT

There are two apparently contradictory conceptions of molecular weight in vogue at present. The chemist determines gram-molecular weight from the vapor density or from the change of the vapor pressure of a solution. For him molecular weights are specific and vary discontinuously. The gram-molecular weight of oxygen is 32, of nitrogen 28, and of hydrogen about 2.0. Even when gram-molecular weights appear to vary continuously, as they do in the case of nitrogen peroxide or of sulfur vapor, the chemist concludes, very properly, that he is dealing with a mixture of two or more substances. When the apparent gram-molecular weight of nitrogen peroxide varies continuously between 92 and 46 as limits, the chemist accounts for this by postulating varying mixtures of N_2O , and NO_2 . When the apparent gram-molecular weight of sulfur varies continuously from about 192 to about 64, the chemist postulates continuously varying mixtures of substances to which he assigns the formulas S_8 , S_4 , and S_2 . To get the absolute molecular weight of a substance the chemist divides the gram-molecular weight by the Avogadro number, 6.1×10^{23} or thereabouts, for the number of molecules in one gram-molecular weight. The absolute weight of a molecule of oxygen gas is therefore something over 5×10^{-23} grams.

According to the physicists a particle suspended in a liquid behaves exactly like one dissolved, and may be considered a molecule.¹ Einstein "showed that according to the molecular kinetic theory, the colloidal solutions should give osmotic pressure and diffusion just as ordinary solutions, that there is no difference between a suspended particle and a molecule." If we accept this, and the physicists do accept it, the molecular weight ceases to be specific because a suspended particle of platinum may weigh exactly the same, and therefore have the same molecular weight, as a suspended particle of quartz. Furthermore the molecular weight can vary continuously with the size of the suspended particle.

It is difficult to imagine any two conceptions that are more discordant than these two and yet they can be harmonized very easily if we wish to. The molecular weight of the physicist is the weight of, or is

¹ Svedberg, "Colloid Chemistry," 92 (1924).

a function of the weight of, the actual suspended particle. The molecular weight of the chemist—the gram-molecular weight divided by the Avogadro number—is the weight of the single particle of the gas. In other words the molecular weight of the chemist is the lower limiting value of the molecular weight of the physicist and it is only at the limiting values that the specificity comes in.

This way of looking at things makes a result obtained by Perrin ² a little less startling. "It may be interesting to observe that the largest of the granules [of gamboge] for which I have found the laws of perfect gases followed, are already visible in sunlight under a strong lens. They behave as the molecules of a perfect gas, of which the gram-molecule would weigh 200,000 tons." A perfect gas with a gram-molecular weight of 200,000 tons is a bit staggering to the chemist; but it is not so bad if one says that these suspended particles of gamboge behave as hydrogen gas would if the individual particles weighed, in round numbers, three ten-billionths of a milligram, taking a billion as a thousand million.

Einstein's point of view has the further advantage of enabling us to account for the behavior of certain colloidal solutions before it occurs to some of the people on the other side of the fence to cite these cases against us. If one is going to consider the suspended particles in a colloidal solution as forming a second phase in the ordinary sense of the word, they ought to act like a phase of constant concentration, whereas they do not. Tannin in water forms a colloidal solution without any question; but the amount of tannin adsorbed by a textile fiber from a colloidal solution of tannin varies continuously with the apparent concentration of the tannin. This is exactly what should happen if the suspended particles of tannin can be considered as equivalent in certain respects to a solution of tannin. The substantive dyes are all in colloidal solution; but the amount taken up by cotton varies continuously with the apparent concentration of the dye. In other words we get the same general form of isotherm for adsorption from a true solution and from a colloidal solution.

The difficulties about hemoglobin and oxygen now disappear. It is practically certain that hemoglobin and oxygen form a definite compound. The experimental data seem to show that this compound dissociates much as it should if the hemoglobin and the oxygen compound were both in true solution and yet we know they are not. Bayliss ³ has put the difficulty very clearly. "It may occur to the reader that there is one class of cases of which no mention has yet been made, namely, the taking up of gases by surfaces such as that of charcoal, adsorption, in which we certainly get a relation between the amount taken up and

² "Brownian Movement and Molecular Reality," 46 (1909).

³ "Principles of General Physiology," 619 (1915).

the pressure. This was in fact suggested by Wolfgang Ostwald⁴ as applying to the hemoglobin-oxygen system. But it is obvious that it is very difficult to reconcile the fact that one molecule of hemoglobin, when saturated combines with one molecule of oxygen and no more, with anything but a chemical compound as the final result. The key to the puzzle will probably be found in a combination of the two processes. The amount of oxyhemoglobin would be determined by the amount of oxygen adsorbed on the surface of the hemoglobin under a given pressure. At the same time, there are difficulties in the treatment of the problem from this point of view; but it has, as yet, received little attention. It seems clear that it is not permissible to use either the law of mass action or the phase rule as applying to the case, until it has been proved that they do or do not hold in the case of colloidal solutions, where there must be surface phenomena intervening, although these phenomena may not be as simple as when larger and flatter surfaces are concerned."

If we adopt the Einstein point of view, the mass law does apply to colloidal solutions, so that difficulty disappears. On the other hand the molecular weight of hemoglobin, as defined by Einstein, will vary with the state of aggregation and may therefore vary with the nature and amount of electrolyte. It is quite probable, though not yet proved, that this may account for the peculiar changes in the dissociation equation.⁵ "The form of the dissociation curve is very sensitive to the concentration of hydrogen ions, so that it can be used as an indicator for changes in this direction occurring in the blood either as the result of muscular work, of want of oxygen, or in pathological states of acidosis.

"Now what are the equations to the curves obtained in the presence of acid or of salts? Since hemoglobin is in colloidal solution and, as we have seen, electrolytes have a powerful effect in causing aggregation of colloidal particles, this phenomenon would naturally be looked for as the explanation. A. V. Hill, on the hypothesis of the aggregation of molecules of hemoglobin causing the reaction to become of a higher order than unimolecular, arrived at an expression of the form:—

$$y = 100 \frac{Kx^n}{1 + Kx^n}$$

where y is the percentage saturation of hemoglobin with oxygen, x the oxygen pressure. This formula, by proper choice of the constants, K and n , was found to apply to the experimental data of several cases taken.

⁴ *Kolloid-Z.*, **2**, 264, 294 (1908).

⁵ Bayliss, "Principles of General Physiology," 623 (1915).

"In attempting to understand the meaning of this equation, it is well to point out that Hill himself did not profess to attach any direct physical meaning to the constants, although Barcroft regards K as the equilibrium constant and n as the average number of molecules of hemoglobin in each aggregate. Hill subsequently adopts this view to a large extent. . . .

"The constancy of n with a particular acid leads Barcroft to make the statement that the action of acid does not lead to change in the number of molecules in the aggregates, but to a change of the equilibrium constant. But as we have seen, it is not satisfactorily shown that n refers to the number of molecules in the aggregates, and I might venture to point out that constancy of the exponent is also a characteristic of adsorption."

While it is clearly useful to treat a colloidal solution as having some of the properties of a true solution, it would be a serious mistake not to distinguish between the two at other times. There is nothing inconsistent about this. So far as the effect on the partial pressure of water vapor is concerned, we may consider the alcohol in a dilute aqueous solution as behaving like a gas; but, if we consider the density of the solution or its solvent action for sodium chloride, sugar, or naphthalene, we have to treat the alcohol as a liquid. In fact, if we consider the effect of the water on the partial pressure of the alcohol, we treat the alcohol as a liquid and the water as a gas. When we say that a solute behaves in certain respects like an ideal gas, we do not mean either that it behaves in all respects like an ideal gas or that it is an ideal gas. The fact that many physical chemists do commit this error does not make it any less an error.

If we shake up clean carbon black with water, we get a suspension and everybody knows that the carbon black is not dissolved in the water. The problem is to determine at what point of dispersity a sol changes to a solution. So far, nobody has tried to draw the line sharply; but it can be done if we make one assumption, the accuracy of which will have to be left to the future.

The simpler cases can be decided without making any assumption, by harking back to Gibbs. According to Gibbs the properties of a phase containing any number of components are dependent only on the temperature, pressure, and the concentrations, provided we are working under conditions in which we can ignore effects due to gravity, surface tension, electromotive forces, etc. Conversely, any apparent phase in which the properties depend on something other than the temperature, pressure, and concentration is not a phase in the sense in which Gibbs uses the term. According to this definition a colloidal gold sol is not a one-phase system because we can fix the temperature, pressure and concentration, and yet the color may be either blue or red

by transmitted light. It does not help to talk about surface tension effects because they would not be an important factor in case we were dealing with a true solution.

By the application of the Gibbs criterion in one form or another we can show that most of the colloidal solutions are two-phase systems; and yet we encounter no difficulty with mixtures of gases, a case which bothers a person who tries to define a phase as being either chemically or physically homogeneous. In the last analysis a mixture of two gases is physically and chemically heterogeneous; but everybody knows it is a one-phase system and that is the way it comes out if we apply the criterion of Gibbs.

The difficulty comes with strictly reversible sols of such substances as tannin and soaps. So far as I know now, they satisfy the criterion of Gibbs and yet are two-phase systems. The only way I see now of handling these cases is to make a definite and explicit assumption as to the properties of gases, leaving it to the future to determine whether the guess is a good one or not.

We know that hydrogen will pass through hot platinum while other gases will not. We know that helium will pass through hot quartz while hydrogen will not. We know that carbon monoxide will pass through hot iron while many other gases do not. We know that hydrogen passes through rubber much more readily than does helium, and that carbon dioxide passes through more rapidly than either. We know that there is no apparent relation between the molecular weights of the substances which pass through rubber and those which do not, whereas there is a very distinct relation between the chemical properties. Practically everybody will agree that these are cases in which the gases pass through the diaphragm or membrane because of solubility and not because of a porous structure. I am making the explicit assumption—for which I have no experimental evidence—that any pore which will let one gas or liquid through will let another gas or liquid through. In other words, there is no such thing as a molecular sieve for gases or vapors.

If this postulate be granted, then any substance which can be filtered out by an ultra-filter is in suspension. This criterion enables us to handle the cases of tannin and of soap. Bechhold⁶ was not able to construct an ultra-filter which was permeable to water and which stopped any substances which all of us agree are in true solution.

When one makes the suggestion that inability to pass through an ultra-filter is a proof of a suspension, the usual answer is that a copper ferrocyanide membrane stops sugar, magnesium sulfate, etc. To meet this difficulty it is necessary to point out the theoretical difference between an ultra-filter and a semipermeable membrane. This is the more

⁶ *Z. physik. Chem.*, **60**, 257 (1907); **64**, 328 (1908).

important because I myself have not been entirely free from error in regard to the semipermeable membrane.

We can have two types of semipermeable membrane, one with a continuous film and the other with a porous one. In the case of a continuous film it is essential that the solvent shall dissolve in the membrane and that the solute does not. With a porous film we shall have a semi-permeable membrane only in case we have strong negative adsorption—adsorption of the solvent and not the solute—and in case the diameter of the pores is so small that the adsorbed liquid fills the pores completely leaving no central channel, through which the solution can diffuse. I had assumed previously⁷ that the copper ferrocyanide membrane was probably a continuous film; but that seems to have been a mistake. With our present views in regard to gelatinous precipitates, a copper ferrocyanide membrane must consist of particles of copper ferrocyanide with adsorbed water films, which means that it must be a granular membrane showing strong negative adsorption for many aqueous solutions, notably sugar solutions.

An ultra-filter is essentially a porous membrane which will, by hypothesis, never become a semipermeable membrane unless there is strong negative adsorption. It was a most fortunate accident that Bechhold worked with a material which gave no appreciable negative adsorption and consequently did not change to a semipermeable membrane as the pores were made smaller and smaller. If he had worked with materials having the properties of those used by Bigelow⁸ and by Bartell,⁹ it might have been a long time before we realized the fundamental difference between a semipermeable membrane and an ultra-filter.

The conclusion that a substance which can be filtered out through an ultra-filter is in colloidal solution and not in true solution rests on the explicit assumption that any gas or vapor will go through any pore through which any other gas or vapor will go. It is not known whether this is true or not; but it seems plausible and it gives a definite mark to shoot at, whereas one great trouble in the past has been that people were not willing to put forward any definite criterion which would enable us to decide whether we were dealing with a true solution or a colloidal one.

For instance, Mellor¹⁰ says that "while a solution in equilibrium can be said to have the same composition in all its parts, so that it cannot be separated by mechanical or physical operations into different individual parts, yet, according to the molecular theory, there must be

⁷ Bancroft, "Applied Colloid Chemistry," 110 (1921).

⁸ *J. Am. Chem. Soc.*, 29, 1676, 1675 (1907); 31, 1194 (1909).

⁹ *J. Phys. Chem.*, 16, 659 (1911); 16, 318 (1912); *J. Am. Chem. Soc.*, 36, 646 (1914); 36, 1029, 1036 (1916).

¹⁰ "Treatise on Inorganic and Theoretical Chemistry," 1, 515 (1922).

a limit to the subdivision beyond which the solution can no longer be regarded as homogeneous. Consequently, there is no clearly defined line of demarcation between heterogeneous and homogeneous mixtures. A so-called homogeneous solution, for instance, can "sometimes be separated into its component parts by certain membranes, just as a mixture of gases can sometimes be separated into its constituent parts by atmolysis. A homogeneous solution, or a mixture of gases, however, is considered to be a homogeneous one-phase system because diffusion maintains one uniform concentration throughout its mass."

The converse of the general proposition is not necessarily true that any apparent solution which will pass through the finest ultra-filter is necessarily a true solution. It seems conceivable that an electrically-stabilized emulsion might pass through any ultra-filter. Such a case need not bother us, because it would not satisfy the criterion of Gibbs.

The general results of this article may be summarized as follows:—

1. The molecular weight of the chemist is a function of the vapor density of a gas or of the osmotic pressure of a solution.
2. The molecular weight of the chemist is specific and varies discontinuously.
3. A suspended particle behaves in some respects like a substance in true solution. The molecular weight of the physicist is a function of the weight of the suspended particle.
4. The molecular weight of the physicist is, or may be, non-specific and varies continuously.
5. The two conceptions of molecular weight are not inconsistent if we remember that the molecular weight of the chemist is a function of the weight of the ultimate particle and that the specificity shows only when we get down to this value in any given case.
6. The conception that a colloidal solution may behave in some respects like a true solution enables us to account for the adsorption isotherms with tannin sols or with sols of the substantive dyes. The suspended particles do not behave like a phase of constant concentration.
7. In certain respects a colloidal solution is fundamentally different from a true solution.
8. According to the criterion of Gibbs an apparent phase is not a one-phase system if the properties are not defined absolutely when the temperature, pressure and concentrations are fixed.
9. An ultra-filter is a porous membrane showing no marked negative adsorption—preferential adsorption of the solvent.
10. An ultra-filter, as defined, differs fundamentally from a semi-permeable membrane.

11. The explicit assumption is made that any gas or vapor will pass through any pore through which any other gas or vapor will pass.
12. A substance which can be filtered out by means of an ultra-filter is not in true solution.

Cornell University,
Ithaca, N. Y.

SOME NEW ASPECTS OF THE SURFACE TENSION OF COLLOIDAL SOLUTIONS WHICH HAVE LED TO THE DETERMINATION OF MOLECULAR DIMENSIONS

BY P. LECOMTE DU NOÛY, Sc.D.

Time-Drop of the Surface Tension of Colloidal Solutions. It hardly seems necessary to speak in detail about the phenomenon on which this work is founded, and which I first described in April, 1922, namely, the slow decrease of the surface tension of colloid solutions. However, the fact that the first papers appeared in a medical journal, the *Journal of Experimental Medicine*, prevented a number of research workers in the colloidal field from becoming acquainted with it. Nevertheless, our instrument and technique were used and the phenomenon was mentioned at the first Colloid Symposium by Wilson and Ries in their paper, "Surface Films as Plastic Solids," quoting Mr. Charles B. Dick's experiments. Up to that date, as far as I could learn, the adsorption in the surface layer was considered as almost instantaneous, except in the case of sodium oleate at high concentrations. By using a technique which permitted the measurement of the static value in a few seconds, it was possible to follow the phenomenon step by step and to show that even at very high dilutions, between 1/100,000 and 1/1,000,000, all the colloidal substances which were dealt with lowered substantially the surface tension of water, as a function of time; in other words, that the static value was lower than the dynamic value. Even those that were supposed to have a weak action on the surface tension, such as proteins, were found to lower it by as much as 20 dynes at a dilution of 1/140,000, under certain conditions. In some recently published text-books or papers (1923), I have just read that such substances as albumin and gum arabic *increase* the surface tension of water. Of course, the measurements were made with the stalagmometer. For gum arabic, we obtained the following figures (Table 1), which we have not yet published as they were of no particular interest. Albumin will be dealt with later.

Recently, a new technique was developed for the study of this phenomenon, which we have termed the "time-drop." The technique previously used consisted of the following:

The solution was poured into the watch-glass and stirred with a small glass rod. As soon as possible afterwards, the table supporting the watch-glass on the du Noüy tensiometer was raised, the surface of

the liquid was brought in contact with the platinum ring, and the reading was immediately taken by means of the torsion wire. The whole operation lasted approximately 20 seconds. Then, the table supporting the solution was lowered slightly, the platinum ring was washed, flamed, put back in place, and 2 or more minutes, according to the nature of the experiment, were allowed to elapse. At the end of the allotted time, the same procedure was followed except, of course, that the solution was not stirred, and that great care was taken not to disturb the surface of the liquid in establishing the contact with the platinum ring. The reading was made carefully, and the operations were repeated as many times as necessary to obtain the various values of the surface tension of the solution as a function of time over a period of, say, 2 hours.

TABLE I
Gum Arabic

Concentration:	1:50	10 ⁻³	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
Initial value, dynes..	71.0	72.0	73.0	73.0	73.0	73.0
Static value, 2 hours.	59.0	60.0	60.5	64.0	68.0	73.0
Drop	12.0	12.0	12.5	9.0	4.0	0.0

Obviously, there were three serious causes of error in this technique. The first was due to the fact that the same solution was used in all measurements. Its surface was disturbed every time a measurement was made and consequently the successive measurements did not exactly express what the value of the surface tension would have been had the surface not been disturbed by the preceding measurement, made only 2 minutes previously. The second was involved in making a measurement, the surface of the liquid being then so much distorted that it was by no means certain whether this did not change the state of the surface layer and render the condition of rupture quite different from that which would have obtained on an undisturbed surface. The distortion may have increased the distance between the adsorbed molecules, and this so rapidly that there was no time to reach an equilibrium before the film was torn. The third cause of error was the personal coefficient: the value of the reading might be different according to the rate at which the surface film was stretched by the individual performing the test.

In order to avoid these errors, clean standard watch-glasses (standard means having the same radius of curvature) were prepared in a number equal to the number of measurements to be performed, and enough solution was made to fill every one with 2 cc. of liquid. Suppose 10 determinations are to be made, and the watch-glasses are numbered

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1 to 10. No. 1 is filled with the solution while on the table of the instrument, so that the measurement may be taken immediately after the liquid is poured in. The reading of this initial value is, for example, 73 dynes (solution of serum at 1/10,000). Then, water-glass 1 is removed, No. 2 is placed on the table, and filled. The platinum ring is brought in contact with the liquid, and immediately the torsion of the wire is increased by means of the knob, exactly as though a measurement were to be made. However, instead of working the vernier

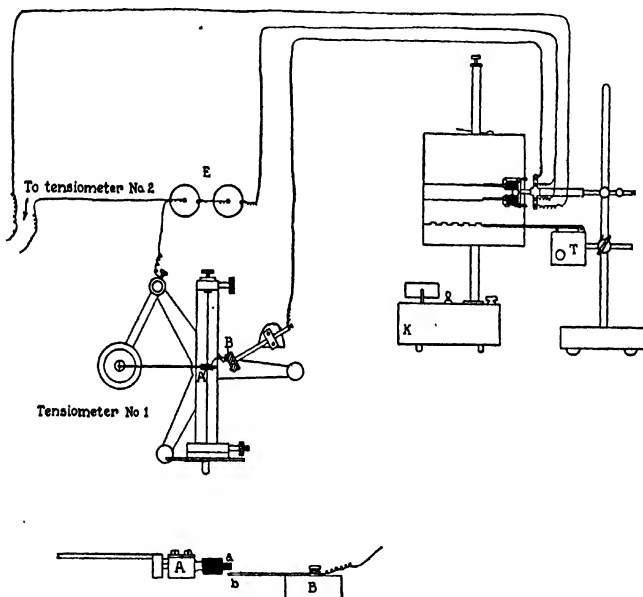


FIG. 1.—Showing arrangement of electric recording device.

up to the breaking point, 73 dynes, it is stopped at 72 dynes and left there. Consequently, the surface of the liquid is raised, but thereafter an opportunity is afforded the surface layer to come to equilibrium, as it is known that, at the moment, it can break only at 73 dynes. As soon as this is done, a stop-watch is set going, and the instrument left to itself. Adsorption in the surface layer takes place normally, reducing the surface tension progressively. As soon as it has fallen slightly below 72 dynes (less than $72-0.05$ dynes), the film breaks, being stretched out by more than an equivalent force (72 dynes), and the time is read on the stop-watch. Some automatic electric recording device, such as that shown in Fig. 1, may be used instead. By

this method the time necessary to reduce the surface tension of the solution by any chosen amount is obtained with great accuracy, the previous causes of error being eliminated. Since the drop in the value of the surface tension is due to the adsorption of molecules from the bulk, the rate at which the surface tension decreases is a function of the adsorption and may be considered as a measure of it. By this technique, curves were obtained which exhibited a greater degree of regularity and smoothness and could be duplicated very satisfactorily. On the whole, they checked with our previous results. The new precision direct reading tensiometers, which were used, are quite sensitive. It happens often that a film will not break for 2 hours, at 55 dynes, for example, when the static value has been reached, while it will break immediately if the tension is raised to 55.1 dynes. In this way, the changes in surface tension can be followed to within $1/10$ of a dyne.

Two tensiometers were used at the same time so as to have a permanent control. The setting of the apparatus and the diagrammatic arrangement of the electric contacts are shown in Fig. 1.

Needless to say, extreme care was taken in the preparation of the solutions, and standard volumetric flasks and standard pipettes were used exclusively. The watch-glasses were always calibrated with a spherometer (reading -7.0 to -7.7), or else Petri dishes were used. All the glassware was cleaned in the usual manner.¹

The results of one set of experiments are given in Fig. 2. Normal rabbit serum was used. As stated above, the rate at which the surface tension decreases as a function of time is a function of the rate of adsorption. At high dilutions the rate of adsorption is low enough to permit the measurement of the surface tension of the solution before the adsorbed molecules have had time to decrease it at all. The readings are identical with those of pure saline solution. Under such conditions, the phenomenon of the decrease of surface tension can be studied closely, step by step, from the very beginning.

At low dilutions the molecules are so close to the surface and so numerous that, no matter how rapidly the first measurement is made (within 1 second, for example), it is impossible to obtain the value of the surface tension before it has been substantially lowered by the solute. In other words, the rate of adsorption, or number of molecules adsorbed in the surface in a given time dt , is very high. In general, it may be said that beyond $1/10,000$, one can always measure a value of the surface tension identical with, or very close to, that of pure saline, that is, study the phenomenon from the beginning; beyond $1/10,000$ and up to $1/2,000$, it happens sometimes that this value is observed, but it depends on the nature of the serum. Below $1/2,000$, it proved impossible to obtain such high initial values, although we succeeded in measuring the surface tension less than $1/10$ of a second after the liquid

had been stirred, by means of the platinum ring itself, in the watch-glass. This explains why at high concentrations the initial value is always low: because the rate of adsorption is considerable; or in other words, because the time required to lower the surface tension of the solvent by 1 dyne is exceedingly small.

At a certain dilution, namely, that which is capable of building up a monolayer (around $1/10,000$) for serum, the initial value of the sur-

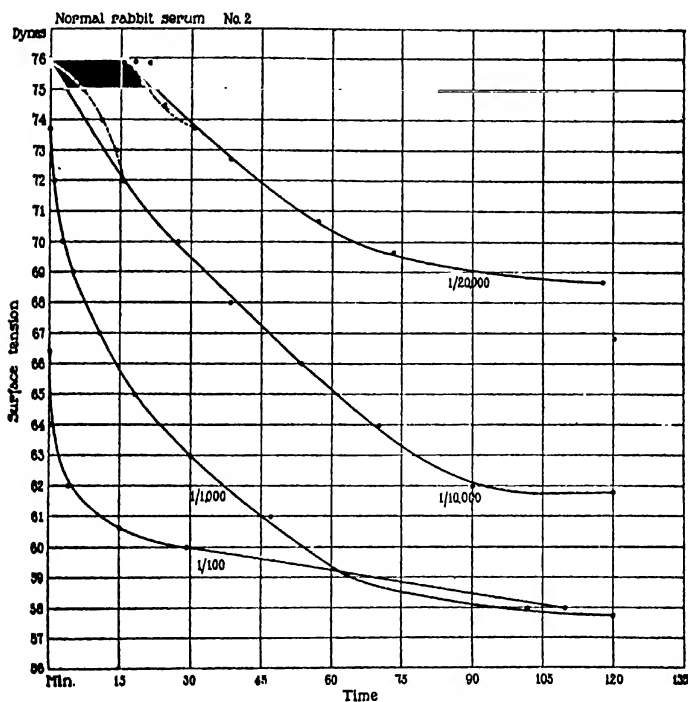


FIG. 2.

face tension is always that of water, or slightly below this value. At a higher dilution, $1/20,000$ for example, in the case of serum diluted with saline solution, the initial value is usually higher, owing to the presence of the salt NaCl (0.9 per cent). The decrease of the surface tension in instances of the latter sort, instead of beginning as soon as the liquid is poured into the watch-glasses, is delayed, and the tension stays constant for some time when suddenly the decrease begins. This can be

explained readily on the basis that a given minimum number of molecules in the surface layer is required in order that the surface tension of the water be lowered by a measurable quantity. As long as the number of adsorbed molecules or groups of molecules is small, they can be considered as floating at the surface and, since no contact exists between them, the stress of the water film is little affected. However, as soon as they cover the whole surface, the drop in surface tension can be measured. As will be seen on the chart, the time necessary to reach

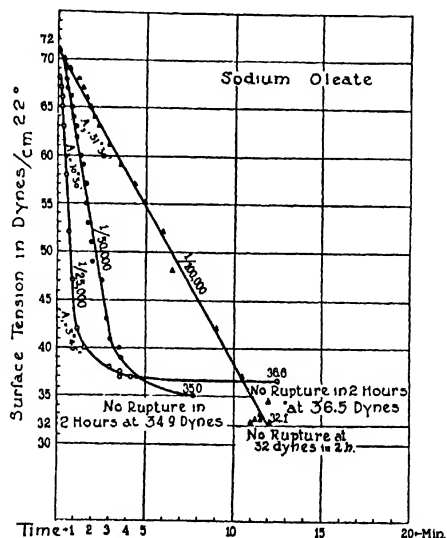


FIG. 3.

this critical moment varies in different experiments. This may be due to differences in the homogeneity of the solution in the watch-glasses.

It is by no means certain that the adsorbed layer only affects the surface tension of water when it is continuous. Instead, very probably, it forms a sort of mesh which becomes more and more solid as time elapses, until the minimum value of the surface tension, characteristic of the concentration 1/20,000, is reached. Even when the equilibrium is established, it is probable that the layer of adsorbed molecules is not continuous. It will be shown that a monolayer of oriented molecules occurs around 1/10,000.^{2, 3} As a 1/20,000 solution is dealt with here, it is possible that even horizontal molecules would not cover the whole surface of adsorption. However, if it is supposed that the adsorbed layer is a continuous monolayer after 2 hours, there can be no doubt

that, at the beginning of the adsorption curve, that is to say, when the action of the adsorbed molecules on the surface tension of water begins to be measurable (Fig. 2), the adsorbed layer is *not* a continuous monolayer. Consequently, when molecules are in solution, in contrast to their behavior when they are not in solution,⁴ they materially lower the surface tension of water long before they have reached the stage of an organized monolayer.

The same technique applied to sodium oleate solutions gave the re-

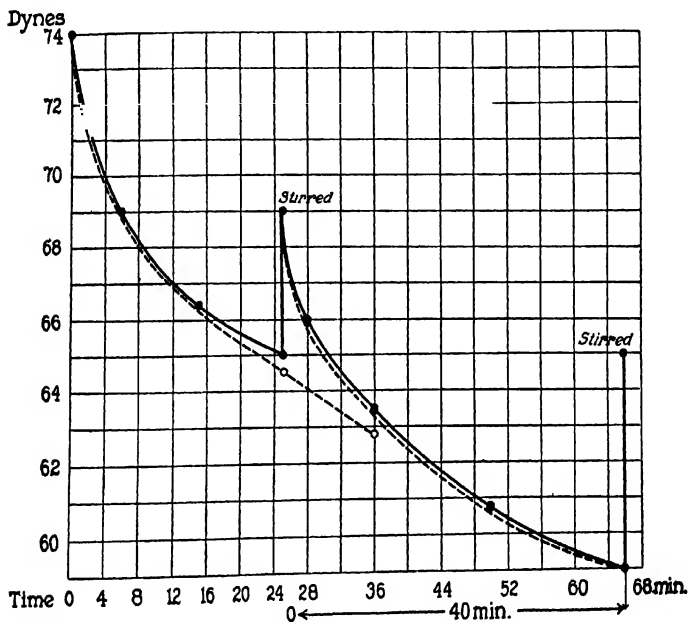


FIG. 4.

sults expressed by Fig. 3. It will easily be seen that, at the concentrations $1/25,000$ and $1/50,000$, the phenomenon is proportional to the time up to a certain point. At $1/100,000$, it remained proportional until the static value was attained, under the conditions of our experiments (watch-glasses containing 2 cc. of solution). It is interesting to note that when the concentration is doubled, the angle of the straight line expressing the drop in surface tension is trebled.

Minimum Values of Static Surface Tension. The curves expressing the static value of solutions of proteins and sodium oleate show at least one minimal value. In the case of serum, the main minimum

always occurs between the concentrations $1/10,000$ and $1/11,000$ (corresponding to an average dilution of proteins of about $1/140,000$). It will be seen later that in the case of the proteins studied (serum, serum albumin, serum globulin, egg albumin), and of sodium oleate, more than one minimum can be observed. In order to explain the first minimum observed, we proposed the hypothesis that it was due to a monolayer, the size and shape of the vessels in which the solutions were exposed being considered. Such a layer is possible at only one con-

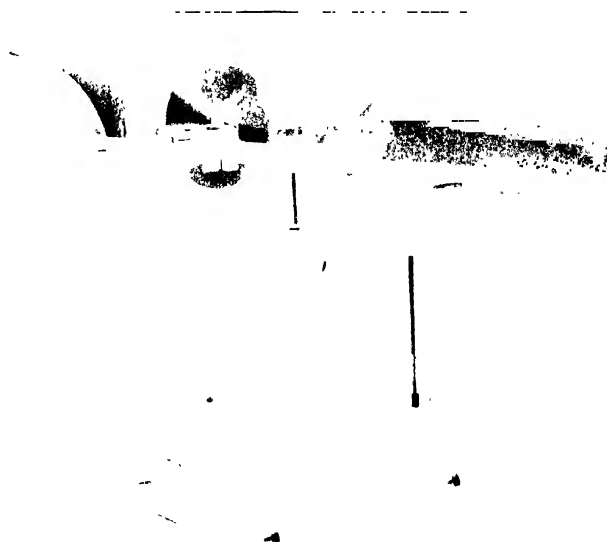


PLATE I.

centration for every orientation of the molecule in the same vessels, and its geometric organization may account for the existence of the smallest possible field of forces compatible with the number of free molecules in the solution. We shall study this phenomenon later.

When the solutions are stirred, even slightly, after standing, the surface tension rises and reaches a value generally smaller than the initial value (Fig. 4). This phenomenon can be repeated a number of times, and for this very reason, great care must be taken when the time-drop is being measured. Special tables were built on which the solutions were kept perfectly immobile, while the tensiometer was

brought successively in front of each solution by means of a small carriage, rolling on rails (Plate I).

Should the above assumption of the existence of a homogeneous oriented monolayer at a given concentration be well founded, the rate of evaporation of such a solution might be slower than that of any other concentration. Water molecules would not escape so freely from a solution covered with such a solid film. At a higher concentration, we may assume that there is a piling up, and no organization of the molecules. Therefore, the water molecules can pass between and escape more easily than through a closely-fitting mesh of identically oriented molecules. At lower dilutions, the breaks in the film would allow the normal process of evaporation to take place. Experiments proved that, with the colloidal solution tested (blood serum), such was the case. The results of a series of experiments are given in Fig. 5.

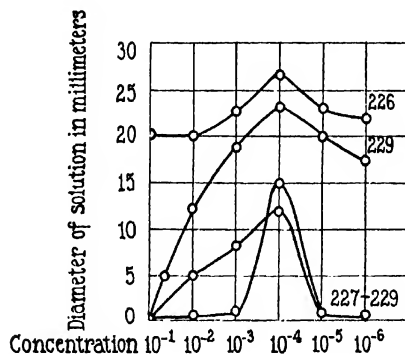


FIG. 5.

So far, our assumption seems to be supported by the facts. It is very plain in these curves that solutions at $1/10,000$ evaporated more slowly than the others. An interesting conclusion may be drawn from these experiments on serum. Of course, the critical concentration 10^{-4} is a function, among other things, of the ratio $\frac{\text{surface}}{\text{volume}}$ of the vessels in which the solutions are exposed. In the case of our watch-glasses, this ratio is about 13, if it is assumed that adsorption takes place on the glass, as will be shown later.

Should the surface increase with respect to the volume (as happens when the volume decreases), this ratio would become very great. If we attempt to calculate the size of the vessels which would require pure serum to develop such a monolayer, in other words, in which the ratio

$\frac{S}{V}$ would be equal to 134,000 instead of 13 (the serum being diluted to 1/11,000), it is found that they would have to be of the order of magnitude of the blood capillaries, filled with blood cells. Thus, perhaps, one of the factors governing the concentration of colloids in the serum is found. According to this assumption, every red blood corpuscle is surrounded by a monolayer of serum proteins. It will be seen later that this layer was found to be 35.4 Angström units thick. Dr. Hugo Fricke came to the conclusion independently, by using an electrical method (capacity of the cells), that this "membrane" must be of the order of magnitude of 30 Å. U. This appears to be a confirmation of our experiments. If it be assumed that our interpretation of the minimum value of the static surface tension of such solutions is a criterion of the formation of a monolayer, it should be possible to compute its thickness, provided it is known whether adsorption takes place on the glass as well as in the free surface. This was ascertained in the following manner:

Two series of watch-glasses were prepared; in one series, 500 very small glass beads were placed side by side. The surface of the glass was thus increased by 14.2 sq. cm. The same set of solutions (rabbit serum at the following concentrations: 1/5,000, 1/6,000, 1/7,000, 1/8,000, 1/9,000, 1/9,500, 1/10,000, 1/10,500, 1/11,000, 1/11,500, 1/12,000, and 1/13,000) was placed in the two sets of watch-glasses.

Without the beads, the ratio $\frac{S}{V}$ equalled 13.2. With the beads, the volume of the liquid being the same, this ratio became 20.3. If our assumption is correct and provided adsorption takes place on the glass, the maximum drop must be shifted towards a higher concentration, and its place will be at a concentration $\frac{20.3}{13.2} = 1.52$ greater, namely, near

1/7,000 instead of 1/11,000. The experiments supported this view entirely, and the maximum drop, as well as the minimum value of the surface tension, occurred at 1/7,000 in the watch-glasses with beads. These beads were, of course, cleaned carefully according to the technique described previously.¹ Fifty of these glass beads were measured with a micrometer caliper, in order to obtain a mean value for their diameter. The sum was 47.68 which, divided by 50, gives a mean diameter of 0.952 mm. The surface of such a bead was 0.02847 sq. cm. which, multiplied by 500, gives 14.235 sq. cm. This surface, added to the existing area of glass (13.33 sq. cm.), brings it up to 27.56 sq. cm. By adding the free surface of the liquid (13.08 sq. cm.), we obtain for

the total surface of adsorption $S' = 40.64$ sq. cm. The ratio $\frac{S'}{V}$ then

becomes $\frac{40.64}{2} = 20.32$. Two control experiments were always carried on at the same time, in watch-glasses without beads. Control 1 was made with the same solution; control 2 with another series of solutions made separately, but from the same serum. The results are given in Figs. 6 and 7.

In a second series of measurements, the volume of liquid was changed, while the free surface remained the same and the glass surface was increased as little as possible. Of course, as the number of

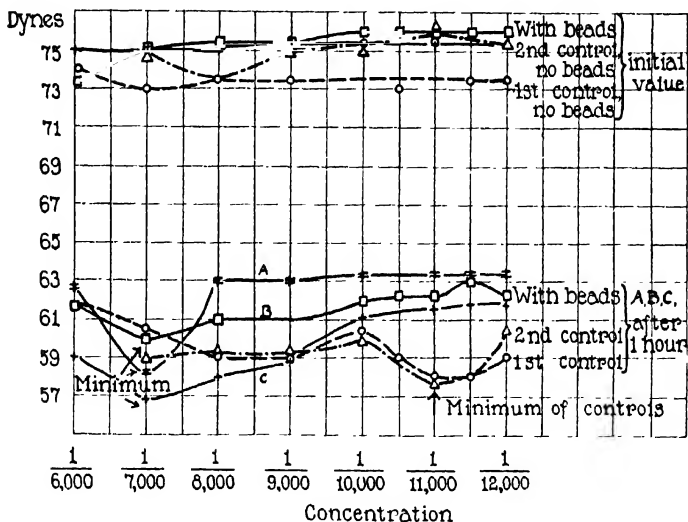


FIG. 6.

molecules was increased (four times as 8 cc. were used), lower concentrations had to be prepared, so that every molecule could find a place on the adsorbed layer, and relatively few molecules could exist in the bulk of the solution.

Petri dishes 4 cm. \pm 0.05 cm. in diameter were chosen. The depth of the liquid, when the dishes were filled with 8 cc. of solution, was about 0.63 cm. The total surface of adsorption, including glass, was 33.1 sq. cm., and the surface of the liquid, as in the watch-glasses, 13.08 sq. cm. If it is assumed that there is adsorption on the glass, the ratio, $R' = \frac{S}{V}$, was equal to $\frac{33.1}{8} = 4.15$, roughly 4. In the other case (adsorption on the free surface only), it was equal to $\frac{13.08}{8} = 1.63$. In

the first case ($R' = 4$), the maximum time-drop should take place at a concentration determined as follows ($R = \text{watch-glass ratio}$ $\frac{26.4}{2} = 13$):

$$\frac{R'}{R} = \frac{C'}{C}, \text{ i.e., } \frac{4.1}{13.2} = \frac{\frac{1}{x}}{\frac{1}{11,000}}, \text{ whence } \frac{1}{x} = \frac{1}{34,200}$$

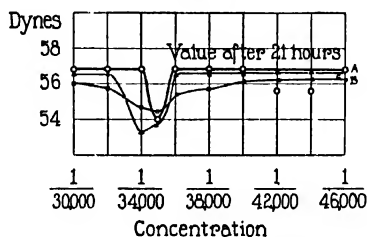


FIG. 7.

In the second case (no adsorption on the glass), the surface being the same as in the watch-glasses, and the volume V being increased

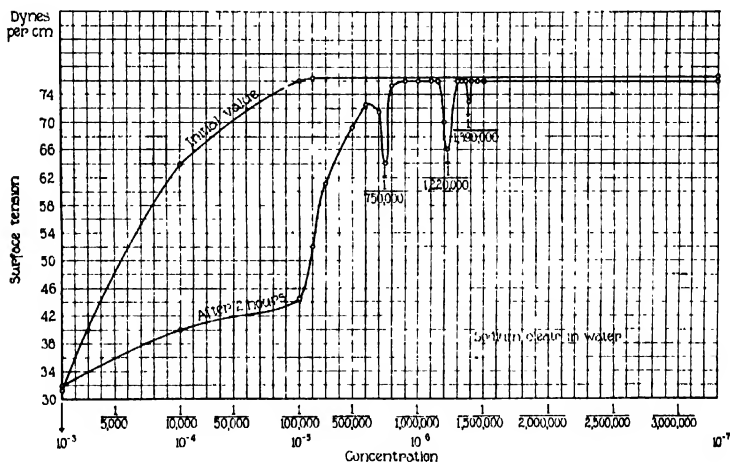


FIG. 8.

fourfold, the concentration should be four times smaller; namely, 1/44,000. Hence, if there is adsorption on the glass, the maximum should occur near 1/35,000; if there is no adsorption, at 1/44,000. As

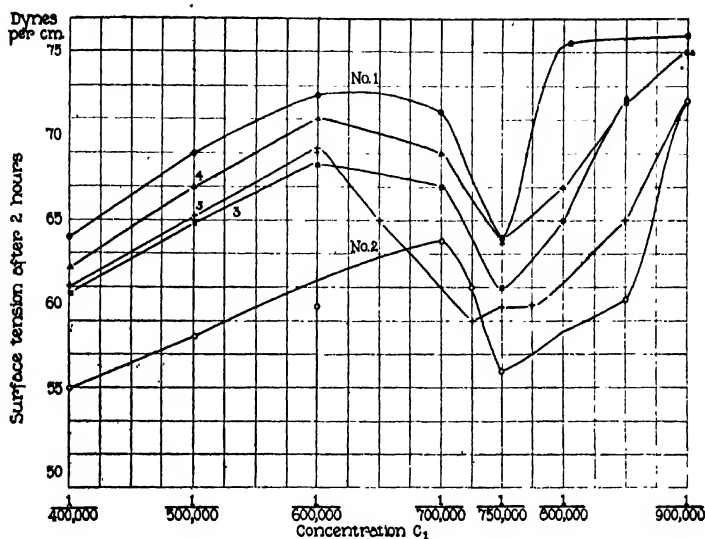


FIG. 9.

the thickness of the liquid was much greater than in watch-glasses, more time was allowed to elapse between the two measurements of surface tension. Proper precautions were taken to prevent evaporation of the liquid.

The results of the readings after 21 hours are given in Fig. 7. A sharp minimum is seen at $1/35,000$, in conformity with our assumption.

The two series of experiments reported above show that adsorption

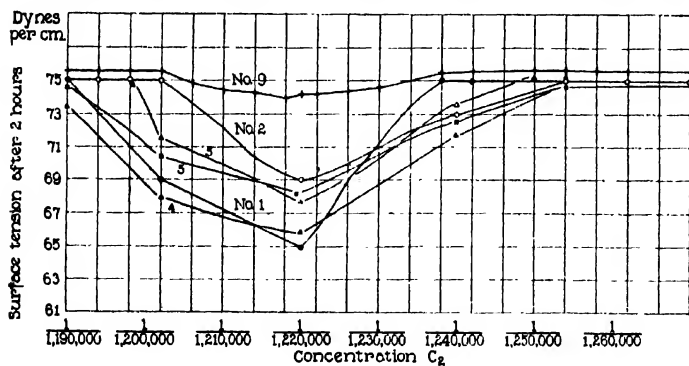


FIG. 10.

takes place on the glass as well as on the free surface. Moreover, as the place of the calculated maxima coincides satisfactorily with the concentration observed, it may be concluded that the orientation of the molecules is probably the same when adsorbed on the glass and on the free surface of the liquid.

It is then possible to compute the thickness of the adsorbed layer by using the following formula:

$$L = \frac{mC}{A\delta}$$

where L = thickness of the adsorbed layer.

m = mass of mixture in watch-glass, always assumed, at $22^\circ\text{C.} = 2 \times 0.9979$ (temperature correction). This is true if density of water is 1 g/cm^3 , and the concentration of the substance very small.

A = area of adsorbing surface (total surface of water in contact with air and glass).

δ = specific gravity of substance in solution.

C = critical concentration at which the minimum is observed.

Experiments with sodium oleate showed three minima: at $1/750,000$, $1/1,220,000$, and $1/1,390,000$, corresponding to the thicknesses of 12.30, 7.56, and 6.64 Å. U.; (the specific weight of sodium oleate was taken as 0.821). From these figures, the number N of Avogadro can be calculated by dividing the known molecular weight 304.35 by the weight

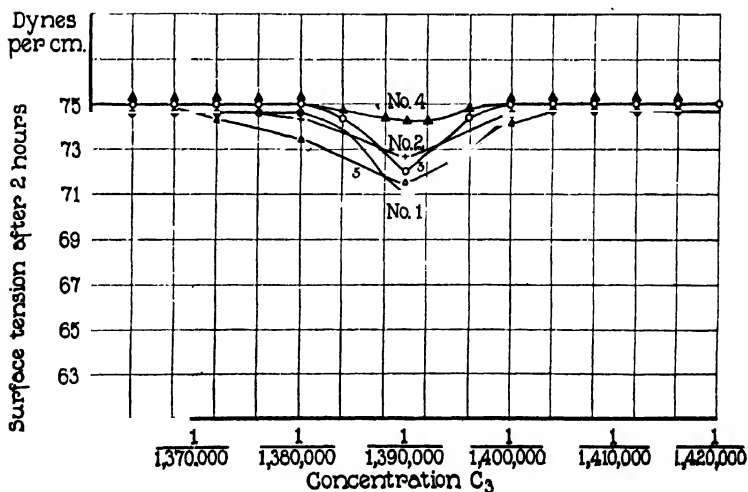


FIG. 11.

of one molecule, in grams, which is 506.91×10^{-24} g. Thus, we found $N = 6.004 \times 10^{23}$, a value which agrees within about 1 per cent with that of Millikan. These dimensions are probably those of the parallele-

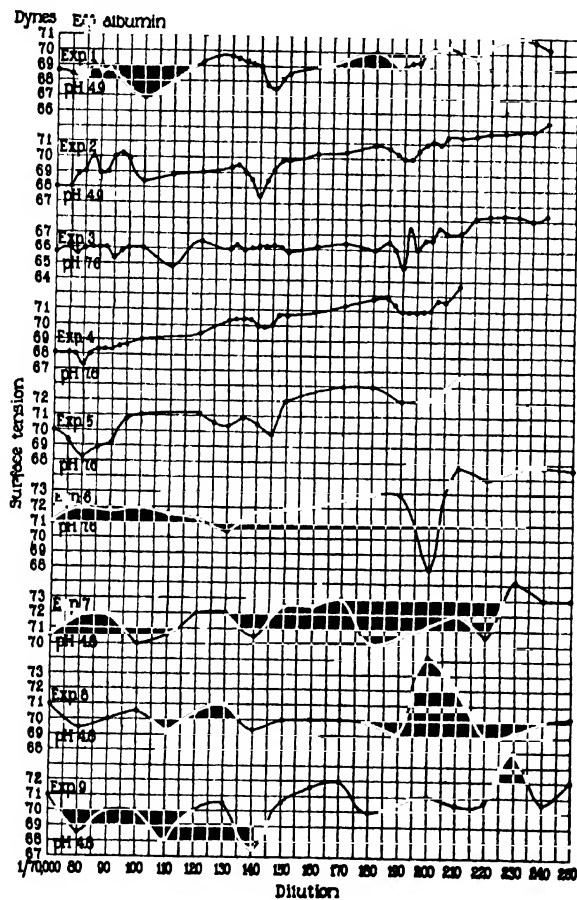


FIG. 12.

pip occupied in space by one single sodium molecule, when surrounded by similarly oriented identical molecules. As this work was published previously in the *Philosophical Magazine* and is about to appear in the *Journal de Physique*, we shall not give further details, but will include the curves representing some of the experiments (Figs. 8 to 11).

However, an interesting fact should be pointed out: a number of different samples of sodium oleate were prepared by Dr. L. E. Baker, according to the classical standard method, with some minor differences and refinements in the details of the technique. But some samples did

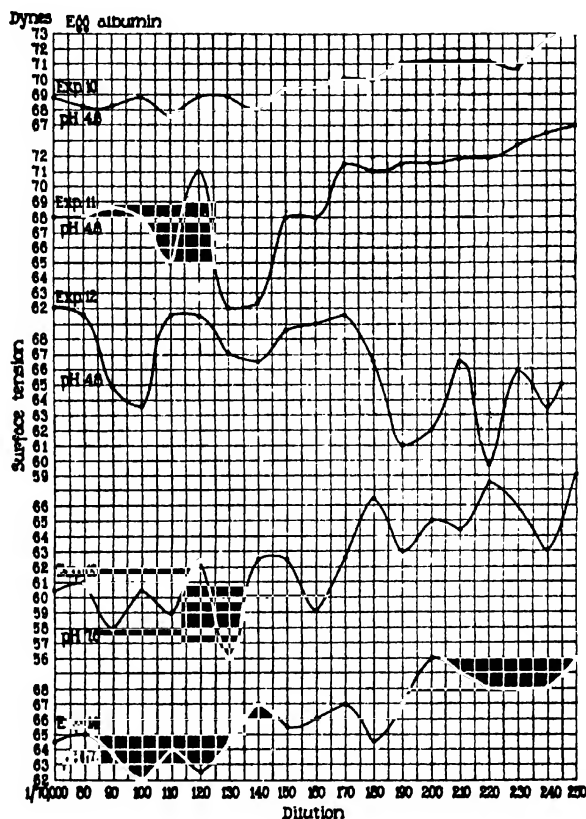


FIG. 13.

not yield a true and stable solution. Instead of remaining perfectly clear for at least 48 hours, these solutions became opalescent almost immediately after their preparation, and no minima could be observed at all. Under the ultramicroscope, a large number of micellæ were seen. This may be due either to an incomplete saponification of the oleic acid, or to the presence of unsaturated fatty acids in the solution, or perhaps to some other cause. It may even be that the presence of a

very minute amount of impurities is necessary to maintain the solution in the state of true solution. Dr. Baker is at present investigating this problem in our laboratory.

In the case of serum proteins and crystalline egg albumin, however, the minima are always present. Over two hundred series of measurements, that is, approximately six thousand, have been made, and the results are always comparable. Figs. 12 and 13 will give an idea of the aspect of the curves. Although they seem somewhat puzzling at first glance, they may be interpreted by plotting the frequency of occurrence of all minima, as in Fig. 14. I do not wish to lay any empha-

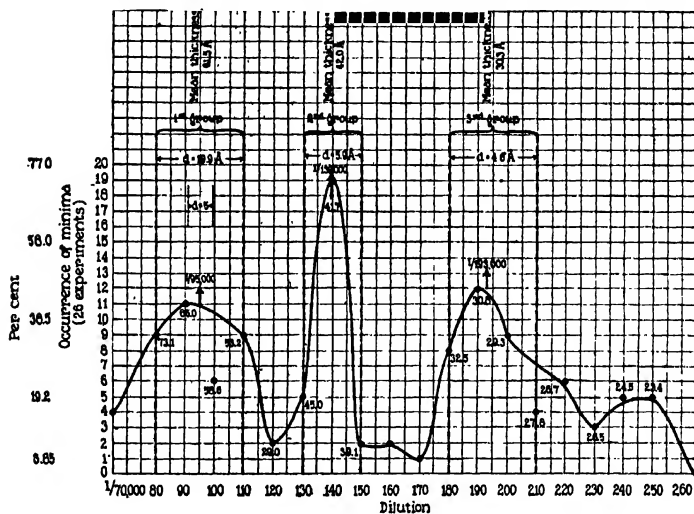


FIG. 14.

sis on the following interpretations, which are only given here as a tentative explanation of the experimental facts. The whole problem has been treated in detail in a paper in the *Journal of Biological Chemistry*, now in press.

If it is assumed that the two principal minima are those occurring at $1/140,000$ and $1/190,000$, and that the group of minima around $1/95,000$ are due to double layers, the dimensions of the "phantom shape" of the molecule considered as a parallelepiped with a square base are $41.7 \times 30.8 \times 30.8$ Å. U. This yields for the upper limit of the molecular weight the value 30,800. If mean values are taken into account, the figure obtained is 30,000. The "mean" values for each group are obtained by multiplying the figures included in this group (Fig. 14) by their frequency of occurrence, and adding them together. This

sum is then divided by the sum of the frequency of occurrence of all minima in that group.

If the shape is not considered a parallelepiped but possibly a prism with a polygonal base, the lower limit is given by assuming that it is a cylinder, the height of which is 42 Å. U. and the diameter 30.3 Å. U., (mean values of groups 2 and 3). The molecular weight then becomes 23,550.

From what is known of the probable molecular weight based on chemical analysis, the first figure appears to be more probable, as it corresponds within about 2 per cent to the double of the value given by chemical analysis, namely, 15,703 ($15,703 \times 2 = 31,406$, instead of 30,800). This would seem to indicate that the molecules do not interpenetrate each other, and that, when packed up in a similarly oriented way in a monolayer, they behave as though they occupied a "phantom space" in the shape of a parallelepiped with a square base. Sørensen, by an osmotic pressure method, gave the figure 34,000 for the molecular weight and, quite recently, Cohn, Hendry, and Prentiss obtained the figure 33,800. Thus, three different methods point to the factor 2 for the figure by which the molecular weight of egg albumin, obtained from chemical analysis, should be multiplied.

Action of Colloids on Crystallization of NaCl. When a solution of NaCl (0.9 per cent) is allowed to crystallize in a perfectly clean watch-glass, the crystals are formed at the bottom and grow as evaporation takes place. They finally assume the appearance shown in the last row of Plate II. When a small amount of any colloid is added to such a solution, concentration in the bulk no longer takes place, large crystals are not formed, and the sodium chloride (or any other salt of the same kind) is deposited on the walls of the watch-glass in a thin, smooth, and often opaque, white layer of very small crystals (Plates III to IX). At the bottom, a darker area with a few tiny scattered crystals indicates that concentration did not take place, and that the molecules and ions are first being adsorbed on the colloidal micellæ, then carried up to the surface layer. As evaporation progresses, the colloidal particles, with their adsorbed NaCl, are deposited on the glass in concentric beaches. At certain concentrations, periodic rings are to be observed; at 1/100, for example, in the case of serum, and 1/1,000 for saponin and sodium oleate. The appearance of the crystals is quite characteristic of the concentration, and varies but little. However, a very interesting phenomenon is observed when sodium oleate is dissolved in a saline solution. At the critical concentrations corresponding to the monolayer, the aspect of the NaCl crystals differs from the others, as seen in Plates X and XI. Thus, in the case of a composite solution of colloids and crystalloids, Gibbs' law, which states that substances tending to increase surface tension will concentrate in the bulk, no longer holds true. This

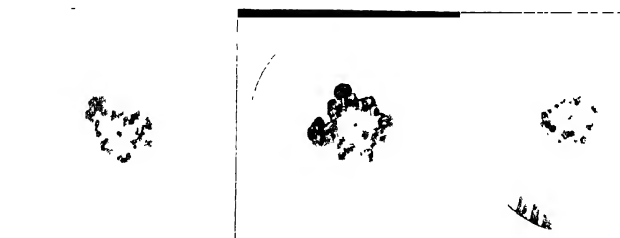


PLATE II.—Pure solution of NaCl (0.9%) evaporated in watch-glasses.

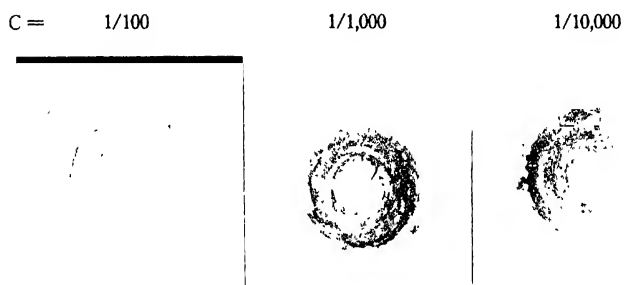


PLATE III.—Rabbit serum in concentrations 1/100, 1/1,000, 1/10,000, respectively, added to 0.9% NaCl solution—evaporated in watch-glasses.

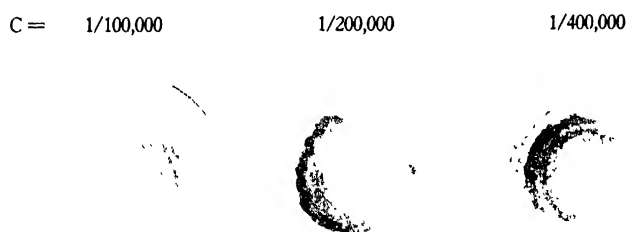


PLATE IV.—Rabbit serum in concentrations 1/100,000, 1/200,000 and 1/400,000, respectively, added to 0.9% NaCl solution.

C = 1/500,000 1/600,000 1/800,000

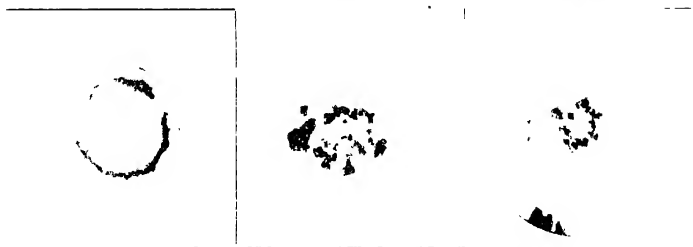


PLATE V.—Rabbit serum in concentrations 1/500,000, 1/600,000 and 1/800,000, respectively, added to 0.9% NaCl solution.

C = 1/100 1/1,000

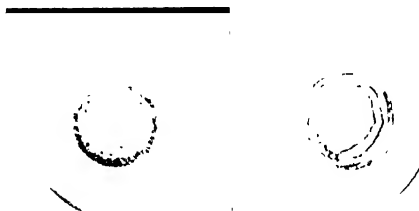


PLATE VI.—Saponin in concentrations of 1/100 and 1/1,000 added to 0.9% NaCl solution.

C = 1/10,000 1/100,000



PLATE VII.—Saponin in concentrations of 1/10,000 and 1/100,000 added to 0.9% NaCl solution.

C = 1/1,000,000 1/10,000,000



PLATE VIII.—Saponin in concentrations of 1/1,000,000 and 1/10,000,000 added to 0.9% NaCl solution.

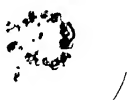


PLATE IX.—Pure 0.9% NaCl solution.

1/1,220,000 1/1,221,000 1/1,222,000



PLATE X.

Sodium oleate in concentrations of 1/1,220,000, 1/1,221,000 and 1/1,222,000 (from left to right), added to 0.9% NaCl solution. The monolayer of horizontal molecules occurred at 1/1,221,000, and, as in PLATE XI, the NaCl crystals show a peculiar aspect, at this critical concentration.

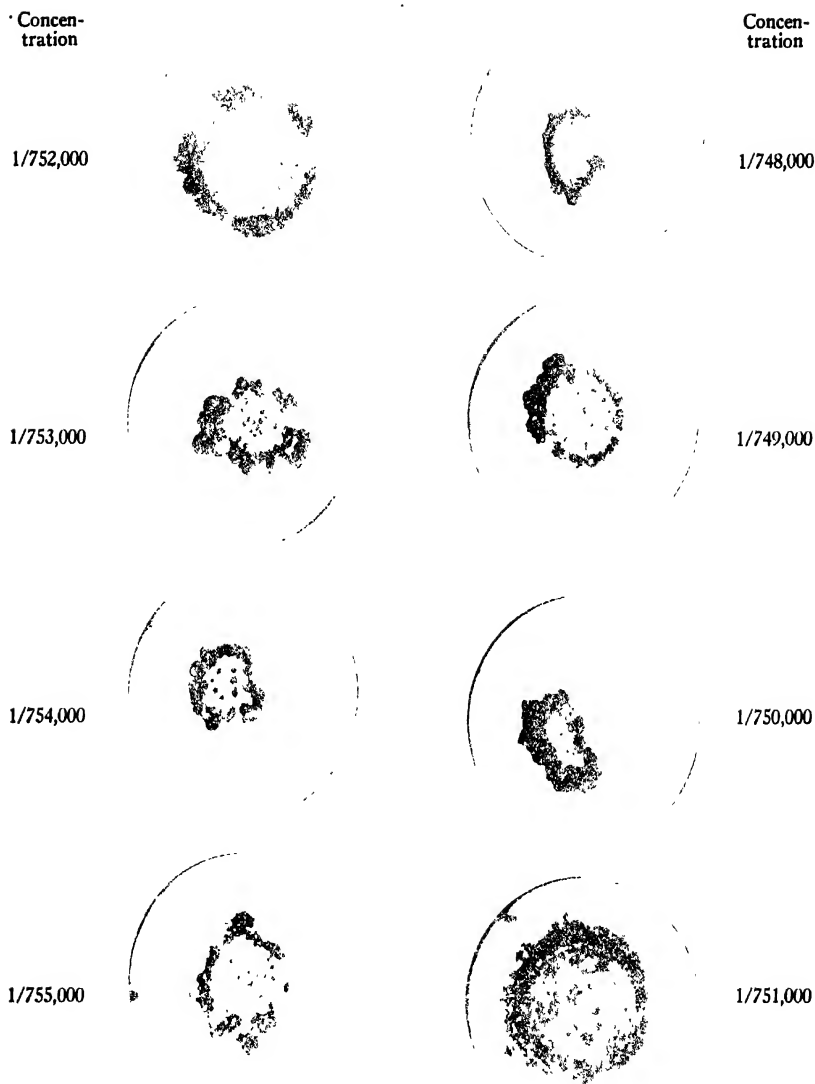


PLATE XI.

Sodium oleate in concentrations of 1/748,000 to 1/751,000 (right row from top down) and from 1/752,000 to 1/755,000 (left row from top down) added to 0.9% NaCl solution. The monolayer occurred at 1/751,000, as indicated by the minimum of the static value of the surface tension. It is obvious that at this concentration, and at the immediately following one, the aspect of the Sodium Chloride crystals is quite different from the others.

observation has thrown light on the spontaneous creation of membranes at interfaces which was difficult to explain before the discovery that not only colloids but also crystalloids concentrated in interfaces at the same time.

Adsorption of One Colloid by Another. When two colloids are present in the same solution, one of the factors governing the final surface tension of the solution is the adsorption equilibrium existing

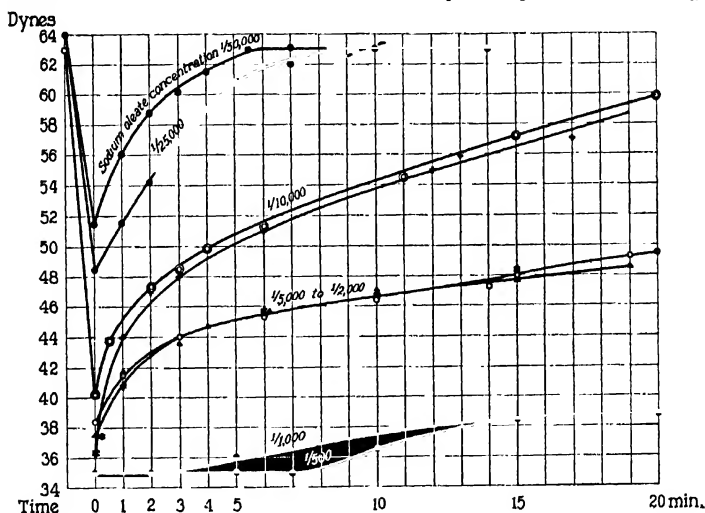


FIG. 15.

between the two. The following experiment will make this clear. If a trace of powdered sodium oleate is added to a less surface-active colloid in solution, such as proteins, gelatin, gum arabic, or metallic sols, the surface tension drops instantaneously by about 50 per cent, then immediately starts rising again until a certain equilibrium (which may be called adsorption equilibrium) is attained. In certain cases (pure blood serum, for example), the final surface tension is equal to the original value before the addition of sodium oleate. In order to observe this phenomenon, measurements must be made every 30 seconds, or at least every minute (Fig. 15).

Laboratories of the Rockefeller Institute for Medical Research,
New York City, N. Y.

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THE DISTRIBUTION AND ORIENTATION OF MOLECULES

BY IRVING LANGMUIR

When thermal equilibrium prevails the distribution of molecules between two regions in which the potential energies of the molecules are different is given in general by the Boltzmann equation

$$\frac{n_1}{n_2} = e^{\frac{\lambda}{kt}} \quad (1)$$

Here n_1 and n_2 are the numbers of molecules per unit volume in Regions I and II and λ is the potential energy which must be expended to move a molecule from Region I to Region II, while T is the absolute temperature and k is the Boltzmann constant¹ 1.372×10^{-16} erg per degree. This equation is closely related to the well known Nernst equation for the electromotive force of a concentration cell.

Equation (1) cannot usually be directly applied to the distribution of molecules between separate phases. For example, if we consider the equilibrium between a liquid and its vapor and let n_2 be the concentration in the vapor and n_1 that in the liquid phase we find that (1) must be replaced by

$$\frac{n_1}{n_2} = \Lambda e^{\frac{\lambda}{kt}} \quad (2)$$

The factor Λ corresponds to the integration constant of the Clapeyron equation for vapor pressures, and its theoretical value must be determined in accordance with the third law of thermodynamics.

A generalized form of the Boltzmann equation has been much used in recent years:

$$\frac{n_1}{n_2} = \frac{p_1}{p_2} e^{\frac{\lambda}{kt}} \quad (3)$$

Here p_1 and p_2 are defined as the *a priori probabilities* of the molecules in the two regions or two states under consideration. These probabilities are frequently dependent upon geometrical factors, but often in-

¹ The Boltzmann constant is merely the gas constant R expressed per molecule instead of per gram molecule.

volve a knowledge of the quantum phenomena accompanying the change in state. Fortunately the factors p_1 and p_2 vary little if any with temperature and, in the case of related phenomena, the values of p_1/p_2 are often nearly alike, or at any rate their variations produce an effect which is usually small compared to that caused by the exponential factor. Thus the generalized Boltzmann equation becomes of great practical value even when we do not possess sufficient theoretical knowledge to determine the value of the probability coefficients.

The ratio of the concentrations n_1/n_2 is also equal to the ratio P_1/P_2 of the actual probabilities per unit volume for the existence of molecules in the two states, so that

$$\frac{P_1}{P_2} = \frac{p_1}{p_2} e^{\frac{\lambda}{kt}} \quad (4)$$

This equation may be applied for example to study the probability of any particular orientation of a molecule in a liquid, with respect to neighboring molecules or to deal with the orientation of molecules in adsorbed films at interfaces between phases. Equation (3) on the other hand may be used in studying the distribution of molecules between phases and interfaces and also in considering the segregation of certain molecules in the neighborhood of others, due to the local fields of force. Debye and Hückel (*Phys. Zeitschr.* **24**, 185, 305 (1923)) for example have recently used the Boltzmann equation to determine the segregation of negative ions around positive ions (and vice versa) and have thus evolved a new theory of electrolytic solutions.

Before we can use Equations (3) and (4) in the way suggested, it is necessary to have definite knowledge of the energy change λ involved in the change of state.

In the case of the molecules of organic substances of non-polar type the so-called physical properties are usually roughly additive. For example, the addition of each CH_2 to a hydrocarbon chain in most compounds containing such chains increases the volume, raises the boiling-point, and alters the solubilities in approximately the same way. It is reasonable to assume, therefore, that the field of force about any particular group or radical in a large organic molecule is characteristic of that group and, as a first approximation, is independent of the nature of the rest of the molecule. For convenience we shall refer to this as the *principle of independent surface action*.

It will be readily recognized that this principle lies at the foundation of the theory proposed by the writer in 1916, according to which the surface tension of pure liquids and the properties of adsorbed films at interfaces depend largely upon the orientation of the molecules in the interfaces.

Thus the surface energy² γ of all the normal saturated aliphatic alcohols is the same as that of the saturated hydrocarbon hexane, namely 50 ergs per cm.² The actual surface energy is that of a hydrocarbon surface in both cases. The alcohol molecules should not be regarded as being packed side by side and arranged with the axes of the hydrocarbon part of the molecule perpendicular to the surface, for there is no force which would compel them to be so arranged. The alcohol molecules which are temporarily in the surface will be free to respond to thermal agitation exactly as if they were in the interior of the liquid, with the single exception that the hydroxyl group cannot itself (for any appreciable fraction of the time) form part of the actual free surface of the liquid. The interaction between the hydroxyl groups of different molecules in the surface is thereby not appreciably altered by the fact that these molecules are forming part of the surface.

The fact of most interest at present is that the hydroxyl group, even in such a small molecule as that of methanol, does not materially alter the surface energy of the CII_3 group which is able to form the actual surface of the liquid by the orientation of the molecule.

The reason that the hydroxyl group avoids the surface must be sought in the energy of the field surrounding this part of the molecule. But by ordinary surface tension and interfacial surface tension measurements we have knowledge of the forces by which hydroxyl groups and hydrocarbon surfaces interact. Let us see whether from such knowledge we cannot determine the energy involved in the orientation of an alcohol molecule and then find from the Boltzmann equation whether this energy is sufficient to cause the orientation indicated by the experiments.

For this purpose let us consider a molecule of methanol. From the density, molecular weight and Avogadro number we find that the volume³ per molecule is 67\AA^3 . Assuming close-packed spheres this gives a molecular surface of 65\AA^2 and a diameter of 4.56 \AA . These figures would not be materially altered if we should assume any other reasonable shape for the molecule. We may take half the surface to consist of the hydroxyl group while the CH_3 radical occupies the other half, these areas being each 32.5\AA^2 .

Imagine a molecule of methanol half immersed in a liquid consisting of other methanol molecules as illustrated diagrammatically in Fig. 1. We use R to denote a hydrocarbon radical and X to denote an active

² The total surface energy γ is related to the free surface energy γ_f by the equation $\gamma = \gamma_f - T \frac{d\gamma_f}{dT}$. The total energy γ is nearly independent of temperature. From the linear decrease of γ_f with increasing temperature it is probable that the variation of γ_f is a direct result of the thermal agitation of the molecules rather than being due to any change in the structure of the surface. The total energy γ is therefore the quantity which will indicate most clearly any orientation of molecules in the surface.

³ We shall express the dimensions of molecules in terms of the Angstrom unit 10^{-8} cm. Areas will be expressed in \AA^2 (10^{-16} cm²) and volumes in \AA^3 (10^{-24} cm³).

group such a hydroxyl. Let us for the present disregard the structure of the liquid and the orientation of the neighboring molecules and consider roughly that half the liquid in contact with the given molecule consists of CH_3 while the other half is OH , as suggested in the diagram. Let us now calculate the surface energy corresponding to the two orientations indicated. If S is the surface of the molecule, γ_R the surface energy of CH_3 and γ_{RX} the interfacial energy between CH_3 and OH radicals then the total surface energy of the molecule in position a is $\frac{1}{2} S \gamma_R + \frac{1}{2} S \gamma_{RX}$ while in position b it is $\frac{1}{2} S \gamma_X + S \gamma_{RX}$. Thus the difference in energy between the two positions which is available for causing the orientation is

$$\lambda = \frac{1}{2} S (\gamma_X - \gamma_R) \quad (5)$$

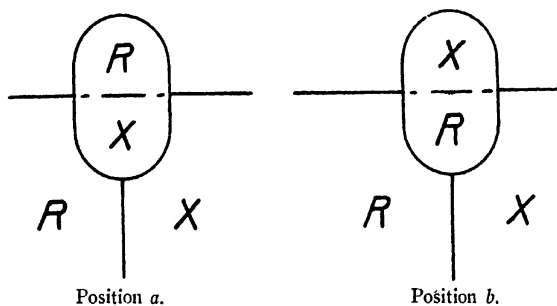


FIG. 1.—Two conceivable orientations of a molecule in the surface of Methanol.

Measurements of the surface energy of hydrocarbons give $\gamma_R = 50$ ergs per cm^2 . The surface energy of water at 20° is 117, but since the water molecules are decidedly polar they will undoubtedly be oriented in the surface so that this value represents the energy of the least active part of the molecule. We shall see from data on the heat of evaporation of water and alcohol that a more reasonable value for γ_X is 190 ergs per cm^2 . Placing $S = 65 \text{ \AA}^2$ in Equation (5) we find $\lambda = 22 \times 10^{-14}$ or 46×10^{-14} erg according as we take 117 or 190 as the value of γ_X .

The degree of orientation of the molecules can now be calculated from the Boltzmann equation (4). The probability coefficients p_1 and p_2 may be taken equal since the geometrical probabilities of the two orientations are the same. Putting $T = 293^\circ$ we find $kT = 4.0 \times 10^{-14}$ erg. Thus the exponent is 5.5 or 11.5 according to the two values of γ_X . The corresponding probability ratios P_2/P_1 are 300 and 100000 respectively.

Thus with the lower value of γ_x the probability of the orientation *a* in Fig. 1 is 300 times greater than that of *b* while with the higher and more reasonable value of γ_x the ratio is 100000 to 1.

A more complete justification of the use of the principle of independent surface action is being published elsewhere.⁴ A brief summary of the principal results will be given here and then further applications will be made, chiefly dealing with adsorbed films at interfaces.

Evaporation of Pure Substances. Consider a molecule AC (Fig. 2) whose surface *S* is composite and let *a* represent the fraction of its surface which is of one kind A (say a hydrocarbon chain) while *c* is

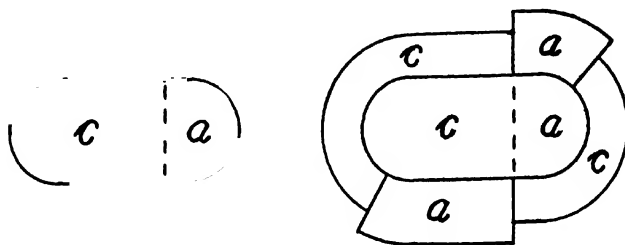


FIG. 2.—Diagram of a molecule AC and of the same molecule in liquid AC.

the fraction of the surface which is of the second kind C (say one or more hydroxyl groups). Then

$$a + c = 1 \quad (6)$$

If we are dealing with a pure liquid each molecule is surrounded by others of the same kind. As a first approximation let us assume that the molecules around a given molecule are neither orientated nor segregated but are arranged in a purely random manner as if no surface energies, λ , were acting. We shall consider later by means of the Boltzmann principle what errors are made by this simplifying assumption.

In Fig. 2 the molecule AC is shown first by itself and secondly, surrounded by others in such a way that the relative areas of the two kinds of surface in contact with each part of the given molecule are in the ratio of *a* to *c*.

The area of that part of the molecule which has an A-surface is *Sa* and of this the fraction *c* is in contact with the C-surface of surrounding molecules. The corresponding surface energy is $Sac\gamma_{ao}$. Where the

⁴ A general discussion of the mechanism and the nature of the forces at the surfaces of molecules and applications of the independent surface action to orientation will be published in the book on Colloid Chemistry being edited by Jerome Alexander. A paper giving a detailed mathematical treatment of vapor pressures of liquids and their binary mixtures and of the mutual solubilities of liquids, together with a comparison with available experiment data, is being submitted to the *Journal of Physical Chemistry* for publication.

A-surface of one molecule is in contact with the A-surface of another there is of course no surface energy.

Similarly the area of contact of the C-surface of the given molecule with the A-surfaces of neighboring molecules is S_{ca} and the corresponding energy is $Sac\gamma_{ao}$. Thus the total surface energy of a given molecule in contact with others is $2Sac\gamma_{ao}$.

Let us now remove the molecule AC to a vapor phase so that it is not in contact with others. Then at the surface of the molecule there is the energy $S(a\gamma_a + c\gamma_c)$ and there is an equal surface energy at the surface of the cavity in the liquid. This latter disappears when the cavity is allowed to collapse but at the same time the new energy $Sac\gamma_{ao}$ appears because of the new interfacial contacts. This energy $Sac\gamma_{ao}$ is half as great as the original energy of the molecule in the liquid, the factor $\frac{1}{2}$ being due to the fact it takes *two* opposing surfaces to make an interface. Thus when the surface S collapses the total interfacial area formed (counting surfaces AA and CC) is only $\frac{1}{2}S$.

The increase in surface energy involved in transferring a molecule from the liquid to the vapor phase is thus

$$\lambda = S(a\gamma_a - ac\gamma_{ao} + c\gamma_c) \quad (7)$$

However if the molecule is a large one it may be so flexible that surface forces tend to make it assume a spherical form when it is in the vapor phase although it may be chain-like in the liquid phase. Also, active groups such as hydroxyl will tend to be drawn into the interior of a large molecule of vapor, just as they are drawn below the surface of the liquid phase. We may neglect the change in S in passing from the liquid to the vapor phase, but may profitably consider that the surface fractions a and c are different in the two cases.

If $a_v S$ and $c_v S$ are the areas of A-surface and C-surface in the molecule of vapor, then $(a - a_v)S$ is the A-surface which becomes buried within the molecule of vapor. Thus we find that the energy for the transfer of a molecule from the liquid to the vapor is

$$\lambda = S[a_v\gamma_a + (a - a_v - ac)\gamma_{ac} + c_v\gamma_c] \quad (8)$$

An analysis of the available data on the heats of evaporation and boiling points of hydrocarbons and alcohols shows that the variation of λ with chemical composition and structure is in excellent agreement with this equation, even in the case of mono-, di- and tribasic alcohols and the compounds having branching chains.

The surface energies γ which give best agreement with these data are

$$\begin{aligned} \gamma_R &= 32.7 \text{ for } \text{CH}_3 \text{ groups} \\ \gamma_R &= 38.2 \text{ for } \text{CH}_2 \text{ " } \\ \gamma_x &= 190. \text{ for } \text{OH} \text{ " } \\ \gamma_{Rx} &= 34 \text{ for the interfacial energy of} \\ &\quad \text{OH against R (hydrocarbon)} \end{aligned} \quad (9)$$

The results show unmistakably the tendency for the larger hydrocarbon molecules of vapor to become spherical and for the hydroxyl group to become partly submerged under hydrocarbon groups in the vapor molecule especially when the hydrocarbon chain is long enough for its end to reach back to the hydroxyl group without too sharp curvature. In molecules containing more than one hydroxyl there is a particularly strong tendency for the hydroxyls to come into contact with each other. The surface energies involved in these changes in configuration are sufficient, according to the Boltzmann equation, to overcome the effects of thermal agitation and probably of the constraints due to imperfect flexibility of the molecules.

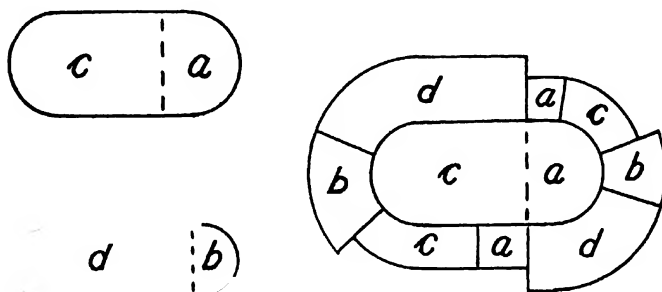


FIG. 3.—Diagram of molecules A and B and of a molecule A in a mixture of A and B.

It is believed that more accurate measurements of vapor pressures of a variety of organic compounds can lead to the development of very definite knowledge of the configurations of their molecules in the vapor phase.

Vapor Pressures of Binary Mixtures. Let us now calculate the energy λ needed to transfer one molecule in a binary mixture to another mixture of the same components having different concentrations. Let us designate the two types of molecules corresponding to the two components by A and B as indicated in Fig. 3. Each molecule is assumed to have a composite surface. The fraction a of the surface of the molecule A is an A-surface, while the fraction c is a C-surface. Similarly b and d are the fractions of the surface of the B molecule which are occupied by B- and D-surfaces respectively, so that

$$a + c = 1 \text{ and } b + d = 1 \quad (10)$$

Let us now consider a binary mixture in which the mol-fraction of A-molecules is A and the mol-fraction of B-molecules is B . The right-hand section of Fig. 3 represents an A-molecule in such a binary mix-

ture. We assume as before that there is neither orientation nor segregation of the molecules which surround a given molecule. Then the relative probabilities that any point on one molecule shall be in contact with an A-molecule or with a B-molecule is proportional to the *total surface areas* of the two kinds of molecules in the mixture. It is thus useful to express the concentrations of the two components A and B in terms of their *surface fractions* which may be defined by the equations

$$\alpha = \frac{S_A A}{S_A A + S_B B} \quad \text{and} \quad \beta = \frac{S_B B}{S_A A + S_B B} \quad (11)$$

where S_A and S_B are the surface areas of the A- and B- molecules respectively. The fractions α and β as well as A and B are related:

$$A + B = 1 \quad \text{and} \quad \alpha + \beta = 1 \quad (12)$$

The surface energy of the A-molecule in the liquid is now found by adding together the energies of each of the five interfaces: AB, AC, AD, BC, and CD. Consider for example the interface AD. The area of the A surface of the A-molecule is aS_A . Of this surface, the fraction β is in contact with B-molecules and of this area of contact the fraction d is a contact with the D-surface. The corresponding surface energy is thus $S_A a \beta d \gamma_{ad}$. For all five terms the surface energy λ_1 is

$$\lambda_1 = S[2\alpha a c \gamma_{ac} + \beta(ab\gamma_{ab} + ad\gamma_{ad} + bc\gamma_{bc} + cd\gamma_{cd})] \quad (13)$$

Now let us imagine that the A-molecule is removed to a vapor phase, leaving a cavity in the liquid. The surface energy λ_2 of the molecule in the vapor phase is

$$\lambda_2 = S(a\gamma_a + c\gamma_c) \quad (14)$$

and the surface energy of the cavity is

$$\lambda_3 = S_A[\alpha(a\gamma_a + c\gamma_c) + \beta(b\gamma_b + d\gamma_d)]$$

When the cavity is allowed to collapse the surface energy λ_3 disappears and new interfaces having the energy λ_4 are produced. The value of λ_4 is found by the summation of 12 terms. The surface of the cavity, before collapse, consists of the areas: $S_A a^2$, $S_A ab$, $S_A \beta b$ and $S_A \beta d$ of the four kinds of surfaces A, B, C and D. Considering that each of these surfaces forms contact with the others in proportion to their respective areas and remembering that it takes two faces to make an interface, we find that the energy λ_4 is

$$\lambda_4 = S_A[\alpha^2 a c \gamma_{ac} + \beta^2 b d \gamma_{bd} + \alpha \beta(ab\gamma_{ab} + ad\gamma_{ad} + bc\gamma_{bc} + cd\gamma_{cd})] \quad (15)$$

The energy λ_{A^V} that must be expended in transferring an A-molecule from the liquid phase to the vapor is then

$$\lambda_{A^V} = \lambda_2 + \lambda_4 - \lambda_1,$$

the energy λ_s dropping out. Substituting into this the values from Equations (13), (14) and (15) and replacing α by its value $1 - \beta$ we thus find

$$\lambda_{Av} = S_A(a\gamma_a - ac\gamma_{ac} + c\gamma_c - \varphi\beta^2) \quad (16)$$

where φ is independent of the concentrations of the components and is a function of the γ 's:

$$\varphi = ab\gamma_{ab} + ad\gamma_{ad} + bc\gamma_{bc} + cd\gamma_{cd} - ac\gamma_{ac} - bd\gamma_{bd} \quad (17)$$

In the case of the evaporation of a pure liquid A we place $\beta = 0$ in (16) and find for the energy of evaporation

$$\lambda'_{Av} = S_A(a\gamma_a - ac\gamma_{ac} + c\gamma_c) \quad (18)$$

so that Equation (16) simplifies to

$$\lambda_{Av} = \lambda'_{Av} - S_A\varphi\beta^2 \quad (19)$$

If we have two binary mixtures (1 and 2) having the same components but in different concentrations, the energy required to transfer an A-molecule from 1 to 2 is, according to (19)

$$\lambda_A = S_A\varphi(\beta_2^2 - \beta_1^2) \quad (20)$$

and in a similar way it may be shown that the energy of transfer of a B molecule from 1 to 2 is

$$\lambda_B = S_B\varphi(\alpha_2^2 - \alpha_1^2) \quad (21)$$

As the first application of these equations let us make a comparison of the energy λ'_A for the transfer of a molecule A from a pure liquid A to a pure liquid B, with the energy λ'_B for the transfer of a B molecule from B to A. In liquid A, $\alpha = 1$ and $\beta = 0$ while in B, $\alpha = 0$ and $\beta = 1$ and Equations (20) and (21) reduce to

$$\lambda'_A = S_A\varphi \quad \text{and} \quad \lambda'_B = S_B\varphi \quad (22)$$

The energy of transfer of molecules A and B between two pure liquids A and B is thus proportional to the surface areas of these molecules.

The Boltzmann equation may now be used to calculate the distribution of molecules between the vapor phase and a binary mixture of liquids. If we let p_A be the partial pressure of the A-molecules in the vapor then by the generalized Boltzmann equation (3) we have

$$\frac{p_A}{A} = Ke^{-\frac{\lambda_{Av}}{kT}} \quad (23)$$

where λ_{Av} is given by Equation (19) and K is a constant into which

are grouped as factors the *a priori* probabilities and the dimensional factors needed to allow us to express the concentrations in the liquid in terms of the mol-fraction A while that of the vapor phase is expressed as a pressure p . We are hereby making the tacit but reasonable assumption that the probability coefficients are independent of the concentrations of the components. The vapor pressure P_A of the pure liquid A is given by (22) if we place $A = 1$ and $\lambda_{A1} = \lambda_{A1}'$ so that

$$P_A = Ke^{\frac{\lambda_{A1}'}{kT}} \quad (24)$$

Dividing (23) by (24) and by combining with (19)

$$p_A = P_A A e^{\frac{S_A \varphi}{kT} \beta^2} \quad (25a)$$

and in a similar way the partial pressure of component B is

$$p_B = P_B B e^{\frac{S_B \varphi}{kT} a^2} \quad (25b)$$

These equations for the vapor-pressures of binary mixtures reduce to the well known Raoult's law when $\varphi = 0$, that is, when the energy of mixing is zero.

Comparison with experimental data, particularly with the accurate data of Zawidski (J. v. Zawidski, *Zeit. phys. Chem.* 35, 129-203 (1900)) shows that even for liquids which depart considerably from Raoult's law these equations, which involve only one adjustable constant φ , usually agree with the data within the experimental error. In the case of mixtures in which S_A and S' differ considerably, the use of these factors and the use of the surface fractions α and β instead of the mol fractions, are found to be thoroughly justified.

The rather meager experimental data available show that in changing from one member of a homologous series of compounds to another, in a given type of mixture, the values of φ change in general in the manner indicated by (17).

Some typical values of φ calculated from published experimental data on vapor pressures of binary mixtures are given in Table I. It is seen that the values of φ are of the order of magnitude of the surface tensions of liquids, but are smaller than these in accord with the fact that in Equation (17) (which gives φ) the coefficients are all less than unity and two of them are negative.

It can be shown from (17) that φ should approach zero if the A molecules and the B molecules become alike in regard to both their values of γ and their surface fractions a and b . We see from Table I

that the observed values of ϕ are very small for mixtures of closely related substances such as ethanol-methanol, benzene-toluene, and methyl acetate-ethyl acetate. The series of benzene mixtures 9 to 15, which are arranged in the order of the ϕ 's, show how ϕ increases as the substance mixed with the benzene becomes less like it, until with methanol ϕ becomes 10 ergs per cm.²

In the case of mixture No. 6, hexane-ethyl iodide, there are only two kinds of molecular surface (R and I) and therefore the 6 terms of Equation (17) reduce to one, so that the value of γ (R-I) can be cal-

TABLE I
"MIXTURE ENERGIES" ϕ FOR VARIOUS BINARY MIXTURES

Mixture	Ref. ^a	Temp. ° C.	ϕ ergs per cm. ²
1. Carbon disulfide-Acetone	Z	35	+ 6.9
2. Water-Pyridine	Z	80	+ 12.9
3. Carbon tetrachloride-Ethyl acetate...	Z	50	+ 1.18
4. Ethyl acetate-Ethyl iodide.....	Z	50	+ 1.90
5. Ethyl iodide-Carbon tetrachloride...	Z	50	+ 0.9
6. Hexane-Ethyl iodide	v. H.	60	+ 2.6
7. Acetone-Chloroform	S	50	- 2.9
8. Carbon disulfide-Chloroform	S	50	+ 2.15
9. Benzene-Chloroform	S	40	- 0.4
10. Benzene-Ethyl ether	S	40	- 0.1
11. Benzene-Toluene	S	40	+ 0.50
12. Benzene-Carbon tetrachloride	Z	50	+ 0.57
13. Benzene-Carbon disulphide	S	40	+ 2.1
14. Benzene-Methyl acetate	S	40	+ 2.5
15. Benzene-Methanol	S	40	+ 10.1
6. Methanol-Ethanol	S	40	0.0
7. Methyl acetate-Ethyl acetate	S	40	+ 0.4

culated from the observed value of ϕ . The three binary mixtures Nos. 3, 4 and 5 involve only 3 components. Because of the symmetry of carbon tetrachloride ($a = 1$, $c = 0$) and the fact that ethyl acetate and ethyl iodide both contain hydrocarbon (R) surfaces, Equation (17) becomes much simplified. By means of the values of ϕ for the 3 mixtures Nos. 3, 4 and 5 we obtain 3 equations containing 5 unknown values of γ . From a study of the surface tensions and other properties of alkyl chlorides and iodides we may conclude that chlorine has properties intermediate between alkyl and iodine. Thus it is reasonable to assume that $\gamma(\text{Cl-I}) = \gamma(\text{R-Cl})$. We have seen in (9) that $\gamma(\text{R-OH}) = 34$ and there are several indications that the relation of -COO- to R is not materially different from that of OH to R. Thus we place

^a Data indicated by Z are those of Zawidzki (l.c.); S are from rough measurements of total pressures of binary mixtures, by G. C. Schmidt, *Zeit. phys. Chem.*, 99, 71 (1921); H. is from H. v. Halban, *Zeit. phys. Chem.*, 84, 129 (1918).

$\gamma(\text{R-C}) = 34$, using C as an abbreviation of the $-\text{COO}-$ group in esters. We then have five equations for our five unknowns and thus find the values given in Table II.

From these values it should be possible to calculate the vapor pressures of any binary mixtures of compounds such as RCI , RI , RCOOR where each R represents any alkyl group. There is also no difficulty in including compounds containing two or more Cl, I or $-\text{COO}-$ groups.

It should also be possible from the principles that have been outlined to develop equations for ternary mixtures and for those containing compounds with molecules having three or more kinds of surface. Thus from the values of γ in Table II we should be able to calculate the partial vapor pressures of a ternary mixture consisting of say propyl-

TABLE II
VALUES OF SURFACE ENERGY γ CALCULATED FROM EXPERIMENTAL DATA ON
VAPOR PRESSURES

Surfaces	Symbol	γ ergs per cm^2
1. Hydrocarbon-Iodine	$\gamma(\text{R-I})$	13.7
2. Hydrocarbon-Chlorine	$\gamma(\text{R-Cl})$	4.1
3. Hydrocarbon- $\text{COO}-$	$\gamma(\text{R-C})$	33.7
4. Chlorine-Iodine	$\gamma(\text{Cl-I})$	4.1
5. Chlorine- $\text{COO}-$	$\gamma(\text{Cl-C})$	17.7
6. Iodine- $\text{COO}-$	$\gamma(\text{I-C})$	16.6

di-chlor acetate, ethylene chloride and amyl iodide, from the vapor pressures of the separate components and the ordinary data of molecular volumes.

Unfortunately there have been very few experimental studies of the vapor pressures of a series of related compounds which can serve at present as a test of this theory.

Measurements are needed of the vapor pressures of binary mixtures of hexane with different simple alkyl compounds such as ethanol, propionic acid, ethyl chloride, etc. From each such mixture studied, a separate value of γ can be obtained and it would then be easy to calculate the vapor pressures of other mixtures of such compounds, and thus check the theory by corresponding experiments.

The boiling points of many binary mixtures of alcohols, bromides, iodides and acetates have been measured by Holley (*J. Amer. Chem. Soc.* **24**, 448 (1902)) and by Holley and Weaver (*J. Amer. Chem. Soc.* **27**, 1049 (1905)). For mixtures containing 0.5 mol fraction of each component the total vapor pressure of the mixture was compared with that of the separate pure components and then by a series of trials the partial pressure and ϕ were calculated by Equations (25).

TABLE III
OBSERVED AND CALCULATED MIXTURE ENERGIES ϕ FOR BINARY
MIXTURES OF ALKYL BROMIDES AND ALCOHOLS

Alcohol	Propyl Bromide		Iso (?)– Butyl–Bromide		Amyl Bromide	
	ϕ_{obs}	ϕ_{cal}	ϕ_{obs}	ϕ_{cal}	ϕ_{obs}	ϕ_{cal}
Methanol	8.90	8.76	7.33	8.52	7.30	8.33
Ethanol	6.97	5.82	6.61	5.58	5.90	5.40
Propanol	5.39	4.52	3.24	4.28	—	—
Butanol	3.43	3.76	2.89	3.53	—	—
Pentanol	3.77	3.21	—	—	—	—

Table III gives a summary of the values ϕ_{obs} , which were thus obtained from the experimental data on mixtures of bromides with alcohols. The values marked ϕ_{cal} have been calculated^o by Eq. (17) from the following values of γ .

Hydrocarbon-bromine $\gamma(R-Br) = 10$ ergs per cm.²

Hydrocarbon-hydroxyl $\gamma(R-OH) = 33.7$

Bromine-hydroxyl $\gamma(Br-OH) = 49.6$

The value of $\gamma(R-Br)$ was assumed to be 10 as a reasonable interpolation between $\gamma(R-I) = 13.7$ and $\gamma(R-Cl) = 4.1$ as given in Table II. The values of $\gamma(R-OH)$ and $\gamma(Br-OH)$ were then determined by least squares to make the agreement as good as possible between ϕ_{obs} and ϕ_{cal} in Table III.

Considering the rather rough nature of these data and the uncertainty in some cases as to whether the normal or iso-compounds were used, the agreement is as good as could be expected. The signs of the differences between ϕ_{obs} and ϕ_{cal} seem to be distributed nearly at random.

It is interesting to note that the value of $\gamma(R-OH) = 33.7$ obtained from these data on the vapor pressures of mixtures of bromides and alcohols agrees with the value 34 given in (9) for γ^{rx} which corresponds to the energy of the same kind of interface, but determined in an entirely different way, viz., from the heats of evaporation of alcohols.

Perhaps the best test of Equation (17) available at present is that based on the published data on the vapor pressures of mixtures of alcohols and water and mixtures of fatty acids and water, although the polar character of these substances undoubtedly causes some mutual orientation and segregation of molecules in these mixtures, so that the assumptions underlying this equation are not completely fulfilled.

^o The surfaces S of the molecules were calculated from the molecular weights and the densities at room temperature, assuming arbitrarily close packed spheres. The surface fractions a , b , c and d were calculated by taking for the surface of the bromine atom $25.A^2$ and for the hydroxyl group $80.A^2$.

Table IV contains the results of calculations based on Wrewsky's data (M. Wrewsky, *Zeit. phys. Chem.* **81**, 1 (1913) and **82**, 551 (1914)) on the partial vapor pressures of aqueous mixtures of methyl, ethyl and propyl alcohols at temperatures of 30° and 50°.

TABLE IV
THE MIXTURE ENERGIES ϕ OF ALCOHOL-WATER MIXTURES

Alcohol	α	ϕ_{obs}	ϕ_{cal}
Methanol	0.460	6.1	6.02
Ethanol	0.359	11.5	11.69
Propanol	0.305	15.1	15.0

ϕ_{cal} is based upon
 $\gamma(R-OH) = 33.7$
 $\gamma(R-H_2O) = 37.4$ and
 $\gamma(OH-H_2O) = -12.6$

The value of $\gamma(R-OH)$ was taken to be the same as found from the data of Table III, and the other two γ 's were chosen to make ϕ_{obs} and ϕ_{cal} agree as well as possible. The fact that the *two* constants could be chosen so as to give such good agreement with the *three* values of ϕ_{obs} is evidence for the applicability of Equation (17) from which ϕ_{cal} was derived.

In these calculations the water molecule was assumed to have a single kind of surface. The value $\gamma = 37.4$ is in reasonable agreement with the value 59 obtained by Harkins for the total surface energy of a hexane-water interface by direct measurement.

TABLE V
THE MIXTURE ENERGIES ϕ OF FATTY ACID-WATER MIXTURES

Acid	α	ϕ_{obs}	ϕ_{cal}
Formic	0.739	-15.0	-13.6
Acetic	0.549	+ 2.5	+ 1.1
Propionic	0.459	+ 8.0	+ 8.5
Butyric	0.402	+13.8	+13.4

ϕ_{cal} is based upon
 $\gamma(R-COOH) = 20.0$
 $\gamma(R-H_2O) = 51.4$
 $\gamma(H_2O-COOH) = -31.2$

The *negative* surface energy $\gamma = -12.6$ for the interface between water and the hydroxyl group is not contrary to known facts and is an expression of the very strong effect of this radical in increasing the solubility of organic substances in water.

Table V gives a summary of calculations based upon Konowalow's data (*Ann Phys.* **14**, 34 (1881)) on the total vapor pressures of mix-

tures of water with various fatty acids. The values of γ found from these data are in reasonable agreement with others of similar nature already found. These data are, however, probably only rough. The known existence of double molecules in the vapors of the lower fatty acids should cause deviations from our theory, but the effect is probably not sufficient to prevent the theory from serving as a useful approximation over a wide range of concentrations.

Mutual Solubilities of Liquids. When two immiscible liquids, A and B, are in equilibrium, each has dissolved some of the other, and the partial vapor pressure p_A from one liquid must be the same as the p_A from the other. Thus, from Equations (25) we obtain the following two equations which express the solubilities of the liquids in one another

$$\frac{A_1}{A_2} = e^{\frac{S_A \varphi}{kT} (\beta_2^2 - \beta_1^2)}$$

and

$$\frac{B_1}{B_2} = e^{\frac{S_B \varphi}{kT} (\alpha_2^2 - \alpha_1^2)} \quad (26)$$

The subscripts 1 and 2 refer to the two phases.

If each liquid is only very slightly soluble in the other, the equations take the simple form

$$A_2 = e^{-\frac{S_A \varphi}{kT}} \quad \text{and} \quad B_1 = e^{-\frac{S_B \varphi}{kT}} \quad (27)$$

If $S_A = S_B$, then $\alpha = A$ and $\beta = B$ and the two Equations (26) can readily be combined to one of the convenient form

$$\frac{1 + \Delta}{1 - \Delta} = e^{\frac{S \varphi}{kT}} \quad (28)$$

where $\Delta = A_1 - A_2 = B_2 - B_1$.

We then find that $\Delta = 0$ or $A_1 = A_2$ at a critical temperature T_c given by

$$T_c = \frac{S \varphi}{2K} \quad (29)$$

Above this temperature the liquids are miscible in all proportions, so that there is only one phase, while at lower temperatures two phases exist.

The values of φ calculated from these equations come out usually somewhat lower (10 to 30%) than those from vapor pressures and are probably less accurate. Undoubtedly, in mutually saturated liquids, especially near the critical temperature, the conditions are favorable

for orientation and segregation of the molecules in the liquids, so that we should not expect our equations for solubilities to hold as well as those for the vapor pressures of mixtures of liquids which mix in all proportions.

Nevertheless, the general agreement between the theory and the experimental data seem very satisfactory, especially if we are willing to consider mainly relative rather than the absolute values of φ .

The effects of the relative surface areas of the molecules indicated by Equations (27) seem to be well verified by the experimental data. Thus, in general, for two substances only slightly soluble in one another, the substance having the molecules of the larger surface shows the lower solubility in the other.

TABLE VI
SOLUBILITIES OF FATTY ACIDS IN WATER

<i>n</i>	Acid	Temp.	Observed Solubility Mol Fraction	<i>a</i>	φ_{obs}	φ_{cal}
4	Butyric	—2°	0.052	0.402	10.5	13.3
5	Valeric	16°	0.0062	0.357	15.0	17.3
9	Nonylic	25°	1.4×10^{-5}	0.272	26.0	25.0
14	Myristic	20°	ca. 10^{-8}	0.198	33.0	31.9

Even when solubilities are large, nearly to the point of complete mixing, the same rule applies. For example, the critical temperature of butyric acid-water mixtures is -2.5°C . At -6°C there are two phases. The water phase contains 0.053 mol fraction of butyric acid, but the acid phase contains 0.76 mol fractions of water. The much greater solubility of the water as compared to the acid corresponds to the smaller surface area of the water ($S_w = 37.8\text{Å}^2$) as compared to that of the acid ($S_a = 115\text{Å}^2$). In fact the difference in solubility is almost exactly what the theory indicates, for the value of φ calculated by Equations (26) from A_1/A_2 , involving the solubility of the acid, is 16.9 ergs per cm^2 while B_1/B_2 , from the solubility of the water, gives very nearly the same value, viz. $\varphi = 9.7$. These are to be compared with $\varphi = 13.6$ from Table V for butyric acid-water mixtures at higher temperature from vapor pressure data.

From the very rough data available on the solubilities of higher fatty acids we obtain the values given in Table VI.

The 5th column gives the surface fraction a corresponding to the carboxyl group whose actual surface is taken to be 45Å^2 . The 6th column gives the value of φ_{obs} obtained by Equations (26) from the observed value of the solubility while the 7th column gives φ_{cal} which is

calculated by Equation (17) with the same values for the γ 's as those already used for the calculations of Table V.

It will be seen that the agreement is reasonable and indicates that the marked decrease in solubility of the fatty acids in water as we pass to the higher fatty acids is fully explained by our theory of independent surface action.

It is important to note that the solubility of water in the fatty acids does not continue to decrease without limit as the length of chain increases. As soon as the solubilities have become small Equations (27) are applicable. As the length of chain increases a approaches zero and ϕ approaches the limiting value 51.4 corresponding to $\gamma(\text{R-H}_2\text{O})$ (see Table V). We see, however, from (27) that S_a , the area of the molecule of acid, continues to increase as the chain lengthens and thus A , the solubility of the acid in water, decreases without limit. On the other hand, S_w , the area of the water molecule, remains constant so that the solubility B , of water in the acid, decreases only because of the increasing ϕ and thus approaches a limiting value as the chain lengthens.

Orientation of Molecules in a Liquid Phase

Thus far we have considered particularly the distribution of molecules between different phases on the assumption that there is no mutual orientation of the molecules within the phases. Although the agreement of our theory with experiment proves that in the case of many liquids this orienting effect is negligible, it is believed that such orientation (and corresponding segregation) is the main cause of deviations which occur with the more polar substances.

For example, the vapor pressures of mixtures of alcohols with water give values of ϕ which are nearly constant or independent of the concentration, except that the values obtained from the partial pressures of water over the mixtures containing more than about 0.5 mol fraction of water give values of ϕ which are much too low. Thus, although the partial vapor pressures of methanol over the whole range agree with the value $\phi = 6.1$, and the partial pressures of water from solutions containing less than 0.5 mol fraction of water also agree with this value reasonably well, the values of ϕ for the higher concentrations of water decrease rapidly, so that with a mixture containing 0.8 mol fraction of water $\phi = -20$. With ethanol and propanol similar effects exist but are much less marked.

In order to form a more concrete conception of the principles which govern the orientation of organic molecules within a liquid, let us consider the following model. The inner circle in Fig. 4 represents a molecule AC, the sector a being the A-surface. The annular space between the two circles represents diagrammatically the surfaces of the molecules

which surround the given molecule AC. In choosing the symbols a, b, c, d to represent the surface fractions we may, without loss of generality, so assign them among the 4 kinds of surface that $b < a < c < d$. When the molecule AC is oriented as shown, with the whole of the B-surface in contact with A-surface, all of the C-surface will be in contact with D-surface and the surface fraction of A-surface in contact with D is $a - b$. Thus the total surface energy is

$$\lambda_1 = S[b\gamma_{ab} + (a - b)\gamma_{ad} + c\gamma_{cd}] \quad (30)$$

Let the molecule AC now be turned so that the A-surface does not come into contact with B but only touches D. Then the total surface energy is

$$\lambda_2 = S[a\gamma_{ad} + b\gamma_{bd} + (c - b)\gamma_{cd}] \quad (31)$$

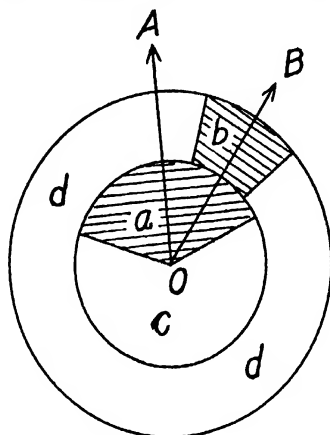


FIG. 4.—Diagram illustrating the orientation of a molecule AC in a liquid BD.

These two orientations correspond to the extreme values of the surface energy; when A and B are partly in contact with each other the energy λ has intermediate values. The difference $\lambda_2 - \lambda_1$ which we shall refer to as λ_0 is thus the greatest surface energy which can be effective in causing orientation.

$$\lambda_0 = Sb(\gamma_{ad} + \gamma_{bd} - \gamma_{ab} - \gamma_{cd}) \quad (32)$$

For convenience let us place

$$\gamma_0 = \gamma_{ad} + \gamma_{bd} - \gamma_{ab} - \gamma_{cd} \quad (33)$$

so that

$$\lambda_0 = Sb\gamma_0 \quad (34)$$

The unique occurrence of b in these expressions is due to the fact that b is the smallest of the surface fractions a , b , c and d because of the convention of nomenclature that we have adopted.

We may represent any given orientation of a molecule AC in Fig. 4 by a given position of the vector OA with respect to the vector OB. If AC passes through all possible orientations we may imagine OA as passing over all points on a spherical surface. We may represent all orientations within any given solid angle by the ratio of the spherical surface described by the vector OA, to the total spherical surface, this ratio being called a surface fraction.

The difficulties in the complete solution of the 3-dimensional problem of orientation are considerable and we may therefore content ourselves with treating it as if it were a 2-dimensional problem. We shall assume therefore that the molecule AC in Fig. 4 has cylindrical symmetry and can rotate only about its axis.

There are three cases to consider.

- I. The molecule is so oriented that the whole of the B-surface is in contact with A. Surface fraction $(a - b)$.
- II. The whole of the B-surface is in contact with C. Surface fraction $(c - b)$.
- III. Part of B is in contact with A while the remainder is in contact with B. Surface fraction $2b$.

We may let P be the probability per unit surface fraction that the molecule may be oriented as in Case I. The total probability that a molecule shall be so oriented is thus $P(a - b)$.

The probability that the molecule shall be oriented in Case II may be found by the Boltzmann Equation (4) and is

$$P(c - b)e^{\sigma}$$

where

$$\sigma = \frac{\lambda_0}{kT} = \frac{Sb\gamma_0}{kT} \quad (35)$$

Within the region corresponding to Case III the surface energy varies linearly between two limits, so that we find the total probability that the molecule shall come under Case III is

$$P \int_0^{2b} e^{\frac{\sigma x}{2b}} dx$$

Now since the molecule must be oriented in one of these 3 ways the sum of these 3 probabilities is unity

$$1 = P[(a - b) + (c - b)e^{\sigma} + \int_0^{2b} e^{\frac{\sigma x}{2b}} dx] \quad (36)$$

If we assume that λ_0 is small enough, σ will be small compared to unity so that we may expand e^σ into a series taking only the terms involving the first order in σ . The foregoing equation thus becomes

$$1 = P(1 + c\sigma) \quad (37)$$

which gives us the value of P .

We may now determine the average surface energy obtained from all possible orientations by weighting each orientation in proportion to its probability. We thus find, taking only terms up to the first order in σ

$$\lambda = \lambda_1 + P\lambda[c + \sigma(c - \frac{1}{2}b)] \quad (38)$$

Since $b < a < c$ we may neglect $\frac{1}{2}b$ in comparison with c . Substituting the value of P from (37) we can reduce Equation (38) to

$$\lambda = \lambda_1 + \lambda_0 c(1 + \sigma a) \quad (39)$$

and by (34) we obtain

$$\lambda = \lambda_1 + Sbc(1 + \sigma a)\gamma_0 \quad (40)$$

This equation allows us to determine theoretically whether the molecules in a given mixture are oriented to an appreciable extent, and if so, we may then estimate the magnitude of the effect produced. Let us consider the following cases:—

1. $\sigma = -\infty$. Equation (40) is inapplicable, but by the method used in the derivation of Equation (36) we see that all the molecules will be oriented as in Case I and the surface energy is thus equal to λ_1 as given in Equation (30). Practically speaking we have this case, if σ is negative and $-\sigma$ is large compared to unity, which occurs if $-Sb\gamma_0$ is large compared to kT .

2. $\sigma = +\infty$. This case is approached if γ_0 is positive and $Sb\gamma_0$ is large compared to kT . The molecules are nearly all oriented as in Case II so that the energy is λ_2 as given by (31) or

$$\lambda_2 = \lambda_1 + \lambda_0 \quad (41)$$

3. $\sigma = 0$. This corresponds to an entire absence of orienting force. The molecules are thus arranged wholly at random. Equation (40) gives

$$\lambda_r = \lambda_1 + Sbc\gamma_0 \quad (42)$$

Substituting in this the value of λ_1 , by (30) and the value of γ_0 from (33) we find

$$\lambda_r = S(ab\gamma_{ab} + ad\gamma_{ad} + bc\gamma_{bc} + cd\gamma_{cd}) \quad (43)$$

Equation (13) was derived as an expression for the energy of a molecule AC surrounded by molecules AC and BD without orientation or segregation. For the case in hand, where AC is surrounded only

by molecules BD, we place $\alpha = 0, \beta = 1$ and find that Equation (13) then reduces to Equation (43) as of course it should.

4. *σ small compared to unity*. This is the case when $Sb\gamma_0$ is small compared to kT . Equation (40) then applies. Let us consider how the energy λ then differs from λ_R the energy corresponding to random orientation. From (40) and (42) we get

$$\lambda - \lambda_R = Sab\sigma\gamma_0 = \frac{S^2ab^2c\gamma_0^2}{kT} \quad (44)$$

This equation enables us to estimate what error we have made in the early part of this paper by assuming a random distribution of molecules in liquids, as for example, in the derivation of Equations (11) to (17).

As a specific example, let us apply Equation (44) to calculate the effect of orientation of ethanol molecules in various mixtures of ethanol with hexane. We have already found that the interfacial energy $\gamma(\text{R-OH})$ is 34 ergs per cm^2 . The surface S_A of ethanol molecules may be taken to be 83.5\AA^2 while that of hexane molecules is 136\AA^2 . The surface S_Aa of the hydroxyl group in the ethanol molecule is 30\AA^2 . With these data we find from Equation (17) $\phi = 4.39$ ergs per cm. for ethanol-hexane mixtures and from this, by Equations (25) we can calculate the partial vapor pressures of these mixtures. We now wish to determine what corrections should be applied to these results to take into account the orienting effect of the ethanol molecules on each other.

We see from Equation (20) that the energy needed to transfer an ethanol molecule from pure ethanol to a mixture is

$$\lambda_A = S_A\phi\beta^2$$

where β is the surface fraction of hexane in the mixture. This expression was substituted in the exponent of the Boltzmann equation to obtain Equation (25a) which would give the uncorrected vapor pressure of ethanol. To get the corrected vapor pressures we should add to λ_A , before making this substitution, the correction given by Equation (44). We place in Equation (33) $\gamma_{ad} = \gamma_{ba} = 34$ and $\gamma_{ab} = \gamma_{cd} = 0$ and obtain $\gamma_0 = 68$. From the value of S_A and S_B we find $a = 0.359$ and $c = 0.641$. Thus Equation (44) becomes

$$\lambda - \lambda_R = 2.72 \times 10^{-14}b^2 \text{ erg} \quad (45)$$

The choice of the value of b is a matter which requires careful analysis. Suppose the mixture is nearly pure hexane containing only a small amount of ethanol. Most of the ethanol molecules will be surrounded only by hexane molecules so that for these molecules $b = 0$. There will be some ethanol molecules, however, whose surface will be in contact with hydroxyl groups of adjacent molecules. For these the

value of b will be determined by the area of contact between two hydroxyl groups.

When molecules are arranged like close packed spheres each is in contact with 12 others, so that the area of contact is $S/12$. We may suppose that about 3 of these 12 regions of contact, for the hydroxyl radical in an alcohol, are occupied by the union with the alkyl group. Thus the surface of 30 \AA^2 , which we have taken as the effective surface of the hydroxyl, represents 9 possible regions of contact, each contact having an area of 3.3 \AA^2 . Allowing for a probable deformation we may thus take the area of contact between adjacent hydroxyl groups to be roughly 3.5 \AA^2 , or 4.2 per cent of the whole surface of an ethanol molecule. Since the surface fraction of the hydroxyl in *pure* ethanol is 0.359 and the surface fraction of each contact is 0.042, we see that there should be on the average $0.359/0.042 = 8.5$ hydroxyl groups in contact with each ethanol molecule. In a mixture of ethanol with hexane in which the mol fraction of ethanol is $A = 0.117$ (*i.e.* $1/8.5$) each ethanol molecule will be in contact, on the average, with one hydroxyl group. For such a mixture we may therefore put $b = 0.042$. Equation (45) then gives

$$\lambda - \lambda_r = 0.0048 \times 10^{-14} \text{ erg} \quad (46)$$

With lower concentrations of ethanol the fraction of the ethanol molecules which are in contact with hydroxyl groups is proportional to the concentration. Thus for these low concentrations we obtain for the average energy correction

$$\lambda - \lambda_r = 0.041 \times 10^{-14} A \text{ erg} \quad (47)$$

which gives the same value as (46) when $A = 0.117$.

For higher concentrations of ethanol than $A = 0.117$ there will usually be more than one contact with hydroxyl. However, these will not in general be adjacent but will be distributed nearly according to the laws of chance. If they were distributed absolutely *uniformly* over the surface of the ethanol molecules there would be no tendency to orient these molecules.

According to the probability laws therefore the effective surface area, $S_{\lambda}b$, of contact with the hydroxyl groups should be proportional to the square root of the number of contacts. Since, by (45) the energy varies as b^2 it is thus directly proportional to the number of contacts or is proportional to the concentration A . Therefore Equation (47) gives the value of $\lambda - \lambda_r$ for concentrations above $A = 0.117$, as well as for concentrations below this value.

In general we may thus assume that $\lambda - \lambda_r$ is proportional to A as in Equation (47) and may place

$$\lambda - \lambda_r = MA \quad (48)$$

where M is the proportionality factor.

Going back to the derivation of Equations (25) from (19), (22) and (23) we may now correct the value of λ_{Av} in (19) by means of (48) obtaining

$$\lambda_{Av} = \lambda_{Av}' - S_A \phi \beta^2 + MA \quad (49)$$

Instead of (24) we then find

$$P_A = K e^{\frac{\lambda_{Av}' + M}{kT}} \quad (50)$$

and (25) is replaced by

$$p_A = P_A e^{\frac{S_A \phi \beta^2 + MB}{kT}} \quad (51)$$

In the example we are considering (ethanol-hexane) the term MB is $0.041 \times 10^{-14}B$ while $S_A \phi \beta^2$ is $3.66 \times 10^{-14} \beta^2$ so the effect of orientation of molecules is practically negligible.

In the foregoing analysis we have considered the orientation of a given ethanol molecule by surrounding molecules which are oriented in random directions. Actually these molecules will tend to be oriented by the given molecule and this in turn would increase the orienting effect on the original molecule. It is probable that value of $\lambda - \lambda_R$ given by (45) or (48) should be approximately doubled to take into account this mutual orientation.

In any case we may conclude that with mixtures of many common organic compounds there will be no appreciable orienting effect and that we are therefore justified in assuming a random orientation as a first approximation. The magnitude of the orienting effect is sufficient, however, for it to become of considerable importance as a cause of deviations in the case of polar substances, and particularly of those whose molecules act as dipoles.

Orientation of Molecules in Interfaces

The example of the orientation of alcohol molecules in a free surface which was considered in the introduction has served to show how the principle of independent surface action may be applied in such cases.

When a sufficiently small amount of a substance such as palmitic acid is placed upon a clean surface of water in a tray the surface tension of the water is not appreciably lowered. But if the free surface of the water is decreased, by sliding a strip of paper over its surface, the surface tension begins to decrease quite suddenly when the free surface becomes about 20 \AA^2 per molecule of palmitic acid. By raising the temperature of the water or by introducing traces of acid into the water, the point at which the surface tension begins to decrease may

occur at an area of 30 to 50 \AA^2 . Such films may be called expanded films.

In 1917 in a discussion [†] of the arrangement of molecules in adsorbed films on the surfaces of fatty acid solutions it was concluded that in dilute solutions "the hydrocarbon chain lies spread out flat on the surface of the water, but as the concentration of the solution increases, the molecules become more closely packed and when the surface becomes saturated, the molecules are all arranged with their hydrocarbon chains placed vertically." The transition between the two states was assumed to take place in the manner that was suggested (on page 1865) for the expanded film of oleic acid, viz., in the expanded film "the molecules are partly reclining on the surface, while in the second case (compressed film) they are packed tightly side by side and are more or less erect upon the surface."

The area covered by a vertically placed palmitic acid molecule is about 20 \AA^2 while the same molecule lying flat on the surface should cover an area of about 108 \AA^2 . Clearly then the expanded films occupying an area of at most 50 \AA^2 must consist of molecules which are only slightly inclined from the vertical. As a matter of fact there is no reason at all why the flexible hydrocarbon chains should orient themselves at any particular angle. They will be quite free to respond to thermal agitation and arrange themselves nearly in the same random manner as in a liquid hydrocarbon, the only restriction upon their motion being imposed by the condition that the *lower end* of each molecule must remain in contact with the underlying water. We can thus readily see why the expanded films are *always liquid* while the contracted or fully compressed films of vertically oriented molecules are frequently *solid*. Taking the volume of the hydrocarbon chain in the palmitic acid molecule as 450 \AA^3 we see that the thickness of the hydrocarbon layer (in \AA units) will be $450 \div a$ where a is the area of the film per molecule (in \AA^2 units). Thus when $a = 20 \text{\AA}^2$ the thickness is 22.5 \AA while for an expanded film for which $a = 33 \text{\AA}^2$ the thickness is 13.6 \AA .

Consider an expanded film as covering a definite area of a water surface. If additional palmitic acid molecules are introduced into the film there is no change in the area of the free hydrocarbon surface. The energy of transfer of a molecule from any given location to an expanded film is thus the same as the energy of transfer of the molecule to an interface between hydrocarbon and water. The total surface energy λ for a molecule of palmitic acid at the edge of a condensed film is -17 while for a molecule in an expanded film λ is -37 . This would indicate that the expanded film should form in preference to the condensed film. However the difference of 20 in the values of λ is not greater than the probable error so that the sign of the difference is somewhat

[†] Langmuir, *Jour. Amer. Chem. Soc.*, **39**, 1888 (1917).

uncertain. In considering the stability of expanded films we must take into account the applied compressive force and the effect of the forces acting between the heads of the molecules which are located in the interface between the hydrocarbon film and the water. To understand these relationships better let us analyze the problem as follows.

Let n molecules of a substance (which spreads on water) be present per unit area as an expanded film on the surface of the water. The flexible hydrocarbon tails of the molecules are subject to little or no constraint except that due to the fact that they are attached to the heads which must remain in contact with the water.

The expanded film may thus be looked upon as a layer of hydrocarbon liquid having at its upper surface a surface energy γ and having adsorbed in its interface with the water n active groups, or heads per unit area. Consider now that by means of a *two-dimensional piston* (for example a paper strip on the surface) the expanded film is allowed to cover only a part of the surface of the water. The spreading force F (in dynes per cm.) exerted by the film is measured by the mechanical force applied to the piston. On one side of the piston is a surface of water which exerts a force γ_w tending to cause the film to expand and on the other side is the expanded film whose *upper surface* exerts a force γ_n while the *lower surface* exerts a force which we may represent by γ_L . Thus for equilibrium we have

$$\gamma_w - F = \gamma_n + \gamma_L \quad (51)$$

If the molecules in the film did not have any active groups, the surface tension γ_L would be equal to the normal interfacial energy γ_{nw} . But the *active groups in the interface* because of their thermal agitation will tend to act like a two-dimensional gas. When these active groups or heads are far enough apart they will exert a force F_L following the gas law

$$F_L = nkT \text{ or } F_L a = kT \quad (52)$$

where a is the area per molecule.

When the molecules are packed as closely as in the ordinary expanded film, we should, by the analogy with the b term in the van der Waals equation, write

$$F_L (a - a_0) = kT \quad (53)$$

where a_0 is the area per molecule for a highly compressed film.

Furthermore the analogy with the equation of state for gases would suggest that when the film is compressed sufficiently, attractive forces between the active groups would come into play and that these might be largely responsible for the small spreading forces observed with some contracted films. In any case, however, we may place

$$\gamma_L = \gamma_{nw} - F_L \quad (54)$$

and thus from Equation (51) we get

$$F = \gamma_w - \gamma_s - \gamma_{sw} + F_0 \quad (55)$$

For very low compressive forces we may neglect the attractive forces between the heads and thus by combining Equations (55) and (53) we get

$$(F - F_0)(a - a_0) = kT \quad (56)$$

as our equation of state for expanded films. Here F_0 is used as an abbreviation for the three γ terms in (55) so that

$$F_0 = \gamma_w - \gamma_s - \gamma_{sw} \quad (57)$$

Thus we see that we should not expect the simple gas law of Equation (52) to hold for expanded films, but such a law should hold only after constants F_0 and a_0 have been subtracted from the observed values of F and a .

Examination of the experimental data on expanded films in my 1917 paper, and in Adam's papers⁸ show that the agreement with Equation (56) is very satisfactory. For large compressive forces deviations occur which are of the kind that are to be expected as a result of attractive forces between the heads of the molecules. For example, Adam's curve (in this 3rd paper) for a film of myristic acid on water at 32.5° C. gives the equation

$$(F + 13)(a - 18) = kT$$

The value of a_0 is thus 18.Å² while F_0 has the value - 13 dynes per cm.

Let us compare this value of F_0 with that calculated by Equation (57). For water at 32.5° the free energy (surface tension) γ_w is 71.0 dynes per cm. The interfacial free surface energy γ_{sw} of octane is 50.4. Since the temperature coefficient is very low the total interfacial energy is about the same as the free energy. Our theory of the structure of the interface leads to the conclusion that the *total energy* is independent of the length of the hydrocarbon chain and since in this case the free energy and total energies are nearly the same the free energy will also be independent of the chain length. Thus for tetradecane (the hydrocarbon corresponding to myristic acid) we may put $\gamma_{sw} = 50.4$.

The free surface energy of octane at 32.5° is 20.4 and the total energy is 48.4. For tetradecane the theory indicates that the total energy will also be 48.4. The free surface energy is a linear function of temperature and becomes zero at the critical temperature. Taking the critical temperature of tetradecane as 680° K we can thus estimate that the free surface energy at 32.5° C. should be 26.8.

⁸ N. K. Adam, *Proc. Royal Soc. A*, **90**, 836 (1921); **101**, 452, 516 (1922); **103**, 676, 686 (1923); **106**, 694 (1924); *Jour. Phys. Chem.*, **29**, 87 (1925).

These values for the γ 's give $F_0 = +0.2$ for octane and $F_0 = -6.2$ dynes per cm. for hexadecane. This latter value does not differ greatly from that calculated from Adam's data on the myristic acid films ($F_0 = -13$). The difference is not greater than should be expected because of the uncertain part played by thermal agitation in the spreading of surfaces.

According to Equation (56) with negative values of F_0 the expanded film should not expand indefinitely even if the compressive force F is made negligibly small. Placing $F = 0$ we see that the maximum area covered per molecule will be

$$a_{\infty} = a_0 - \frac{kT}{F_0} \quad (58)$$

For the case we have considered where $a_0 = 18$, and $F_0 = -13$, we obtain $a_{\infty} = 18 + 32.3 = 50.3 \text{ \AA}^2$ as the maximum area for an "expanded film" of myristic acid on water. This equation shows that the maximum area a_{∞} depends principally upon F_0 . By Equation 57 we see that F_0 is the difference between two relatively large quantities which are nearly equal. A very small percentage change in γ_R causes a relatively large change in F_0 and therefore in a_{∞} . As a matter of fact Adam finds that a_{∞} varies only moderately for different substances. As the length of the chain increases he finds a slight decrease in a_{∞} and considers this a strong argument against the view that the molecules tend to lie flat if they have sufficient room to do so. We have seen, however, by our comparison of octane with tetradecane that γ_R and therefore $-F_0$ increase with the length of the chain, and therefore, by Equation (58), a_{∞} should actually increase as the chain is made shorter. The changes in a_{∞} observed by Adam may be accounted for by very small percentage changes in γ_R .

This theory of "expanded films" requires that the molecules remain in contact while Adam concluded that in such films the "molecules become separated and move about independently on the surface" and that "there are spaces not covered by molecules." Adam considered that the *molecules* of the film behaved as a two-dimensional gas while we conclude that it is only the heads of the molecules that behave in this way.

The fact that a palmitic acid film shows no measurable tendency to expand beyond a certain definite area is in accord with the following values of λ which have been calculated from the γ 's by the principle of independent surface action.

$\lambda_1 = 181 \times 10^{-14}$ erg for a molecule oriented vertically in a water surface with the carboxyl group down and surrounded by water molecules except at its upper end which reaches the free surface.

$\gamma_2 = 64 \times 10^{-14}$ for a molecule lying flat in the surface with the carboxyl group turned downward into the water at one end of the molecule.

$\gamma_3 = 4 \times 10^{-14}$ for a horizontal molecule at the edge of a film of vertically placed close-packed molecules.

$\lambda_4 = -17 \times 10^{-14}$ for a vertically placed molecule at the edge of a film of similar vertical molecules.

The difference between λ_4 and λ_1 or λ_2 is so great that according to the Boltzmann equation no appreciable number of molecules can remain isolated in the surface. The few molecules that do exist as separate molecules, however, must all lie flat in the surface because of the great difference between λ_2 and λ_1 .

If we consider lower members of the fatty acid series we find that the difference between λ for the single molecules and for those in clusters becomes much smaller so that separate molecules do exist and therefore the surface tension of water is decreased by even dilute films of these acids in which a , the area per molecule, is very much greater than in expanded films of palmitic acid. The values of λ prove that in such two-dimensional "gaseous" films the molecules lie flat in the surface.

Research Laboratory,
General Electric Co.,
Schenectady, N. Y.

PHOTOGRAPHIC SENSITIVITY: A COLLOID CHEMICAL PROBLEM

By S. E. SHEPPARD

In a paper presented at the Colloid Symposium of 1923¹ the writer discussed one important colloid chemical factor affecting photographic sensitivity. It was shown that in one and the same emulsion sensitivity increases statistically with the size of grain, that is, the *chance of a grain being made developable by a given exposure increases with the projective area*. When, however, different emulsions are compared, there is no necessary relation between average grain size and sensitivity, so that some other factor or factors must intervene.

Quite a few years ago now² it was found by Lüppo-Cramer that the sensitivity of photographic plates, particularly of ripened ones, could be greatly diminished by treatment before exposure with chromic acid solution, followed by thorough washing to remove the chromic acid. More recently³ it was pointed out by the writer that this desensitizing reaction, particularly when it could be established that the desensitizer was completely removed before exposure to light, might become a valuable criterion for the theory of sensitivity, particularly in deciding between the chemical and physical hypotheses. And in fact a series of investigations in this country and in England have borne this out. It was shown by the writer and his collaborators⁴ that desensitization in this manner was possible with practically every kind of photographic emulsion, both high speed and low speed, and the reaction was shown to be related to the destruction of the latent image by chromic acid and other oxidizing agents. It was further observed that in many instances the effect upon the *latent image*, upon the same material (notably coarse grained high speed emulsions) was relatively greater than upon the *sensitivity*. An important consequence of this was brought out independently by W. Clark.⁵ This investigator showed that preexposure to light greatly increased the efficiency of chromic acid in reducing sensitivity, this being particularly marked with high speed emulsions. (Cf. Fig. 1.)

¹ 1st Colloid Symposium Monograph, p. 346 (1923).

² Lüppo-Cramer, *Phot. Mitteilung*, 1909, p. 328.

³ *Brit. Journ. Phot.*, 1922, p. 61.

⁴ *J. Frankl. Inst.*, 198, 779, 802 (1928).

⁵ *Phot. Journ.*, 63, 280 (1923).

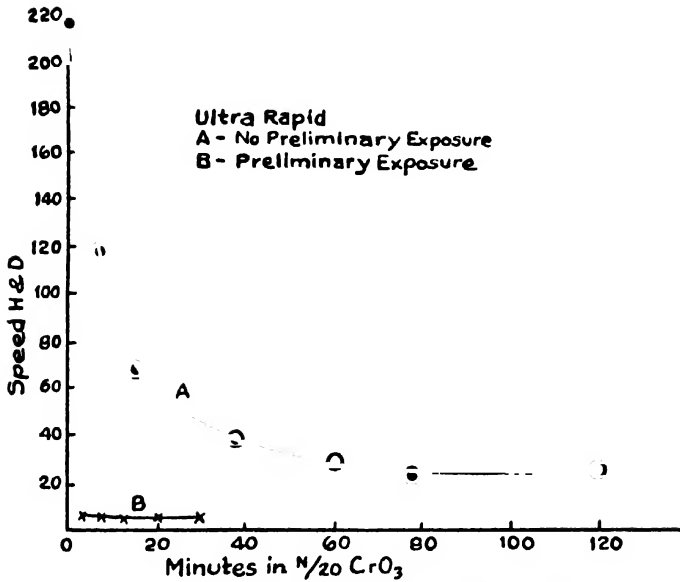


Fig. 1. (From W. Clark. *Phot. Journ.*, 63, 230 (1923).)

It might be concluded from this that the conversion of sensitivity substance into latent image substance facilitates the attack of oxidizing

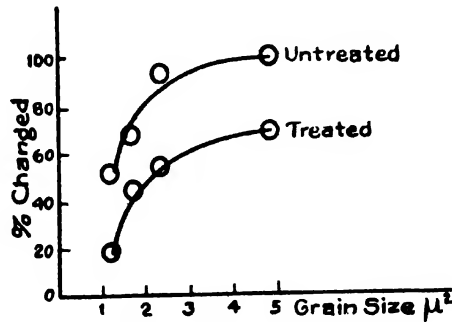


Fig. 2. (From W. Clark.)

agents. Furthermore, it was shown independently by Sheppard, Wightman and Trivelli,⁶ and by W. Clark⁷ that the smaller grains in the same

⁶ *Journ. Frankl. Inst.*, 196, 779, 802 (1923).

⁷ *Phot. Journ.*, loc. cit.

emulsion are relatively more reduced in sensitivity by chromic acid than the larger ones. (Cf. Fig. 2.)

From this it might be concluded that either:

- i) the sensitivity substance is held in different fashion in the larger grains
- or ii) the mechanism of latent image formation is such that removal of the sensitivity substance still further shifts the chance of developability in favor of the larger grains.

I have used here the term "sensitivity substance" without explanation. Let it be said that investigators of these desensitizing phenomena are agreed that there must be present in the silver halide grain a substance other than silver bromide which increases photographic sensitivity and is destructible, in large measure, by chromic acid. I shall use the term "sensitivity substance" to mean this sensitivity promoting material.

Origin of the Sensitivity Substance

It has long been known to emulsion makers that different varieties and batches of gelatin used to prepare the same emulsion could give quite different photographic qualities, and notably different sensitivities. Yet so long as no exact characterization of the grain and grain-size distributions was possible, it could not be stated with certainty whether this was due to an action upon just these factors, in the precipitation and ripening, or to a purely chemical action upon the silver halide. Several years ago the writer had prepared three emulsions with different gelatins, by the same procedure. These had practically identical grain characteristics as shown by the following table and curves. (Cf. Fig. 3.)

TABLE I

No.	Mean Grain Size mm. ² × 10 ⁻⁴		Mean Equiv. Diameter in μ		Standard Deviation mm. ² × 10 ⁻⁴		Speed H. & D.	γ∞	Latitude at γ—0.5
	a	b	a	b	a	b			
1	31.6	31.6	.64	.64	147	147	44	1.58	(50.0)
2	42.2	52.3	.73	.82	166	361	78	1.60	(90.0)
3	35.4	43.6	.67	.75	172	326	340	3.70	(70.0)
4	36.2	41.0	.68	.72	176	286	320	3.64	(65.0)

In this table the *mean* grain size, *mean* diameter and *standard deviation* from the *mean* have been calculated for two cases,

- a. a restricted range, to 30 classes, before gaps in the distribution appeared.
- b. total range, from smoothed curves, hence including filled-in values.

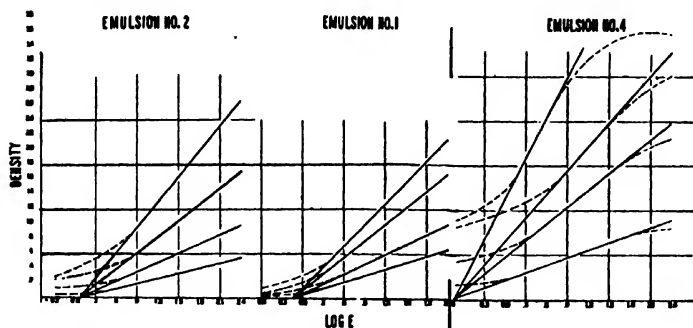


FIG. 3.

With respect to the terms used in Table I in summarizing the grain observations, it may be noted that:

- a. The range signifies the limits of grain size observed, extending in this case from $.8 \times 10^{-8}$ sq. mm. to 700×10^{-8} sq. mm., that is, with these emulsions, a range of 1 to 3500 in areas, or about 1 to 60 in equivalent diameters.
- b. The *mean grain size* is the arithmetical mean of all the observed values; the equivalent mean diameter is calculated from this for a circle of equivalent area.
- c. The *standard deviation* is the accepted measure for the dispersion or "spreading" of the observed values from the arithmetic mean, as calculated by the formula

$$\text{standard deviation } \sigma = \sqrt{\frac{\sum(x^2)}{n}}$$

where $\sum(x^2)$ is the sum of the squares of deviations of the grain sizes from the arithmetic mean, n number of grains measured. As stated, it expresses numerically the spreading or scatter of the observations. The more homogeneous or uniform sized the grains are, the smaller this quantity will be, the less homogeneous they are in size, the greater it will be.

In spite of this the photographic properties, and particularly the average sensitivities (speeds) were quite different. Hence, it might be concluded that the *sensitivity substance is specifically afforded by the gelatin, and in greater degree by one gelatin than another.*

Nature of the Sensitizing Action

One of the first possibilities as to the action of the sensitivity substance is that it might be an optical sensitizer, in this case for the blue violet region of the spectrum. It can be shown that this is not the case.

In the first place, if the substance were an optical sensitizer for the blue violet, it should be possible to take an emulsion of the type described above made with relatively *inert* gelatin, and optically sensitize it relatively highly for the green and red rays, while leaving the blue violet sensitivity very low. But if the normal blue violet sensitivity is *optically* due to the silver halide (in consequence of the absorption of this), and is only catalytically raised by the "sensitivity substance," this would not be possible. That is, the photochemical change of the *optically* sensitized silver halide, including simple undyed silver halide, would be *photographically* sensitized (for development) by the "sensitivity substance," in each case independently. Experiments made by the writer some years since showed that this is what happens. An emulsion made up with *inert* gelatin was divided into four parts. One of these was untreated; of the others, one was optically sensitized with erythrosin and ammonia, another with pinacyanol, for red sensitizing, and a third given a treatment for high green sensitivity. Speeds (H. & D.) were determined, also tri-color ratios, and wedge spectrograms taken. The tests were repeated with the same emulsion (*i.e.* having the same grain characteristics) made with a strongly sensitizing or "active" gelatin. The results showed that in the first case no high green or red sensitivity could be obtained, such as was obtainable in the second case, while the *ratios* of green and red sensitivity to blue sensitivity were substantially the same.

This conclusion, that the "*sensitivity substance*" is *not an optical sensitizer*, was borne out later by entirely independent means. In conjunction with E. P. Wightman and E. E. Richardson, high speed film was desensitized with chromic acid, and the *relative* spectral sensitivity determined in the ultra-violet and blue-violet for both the untreated and the desensitized films.

The plates of which spectrograms are shown in Figs. 4 and 5 were as follows:

TABLE II

Series A and B	Relative Speed
1. Untreated Plate	504
2. Water-washed	650
3. Treated with Chromic Acid	17
4. Treated with Chromic Acid and then with pinakryptol green	5
5. Treated with pinakryptol green	12

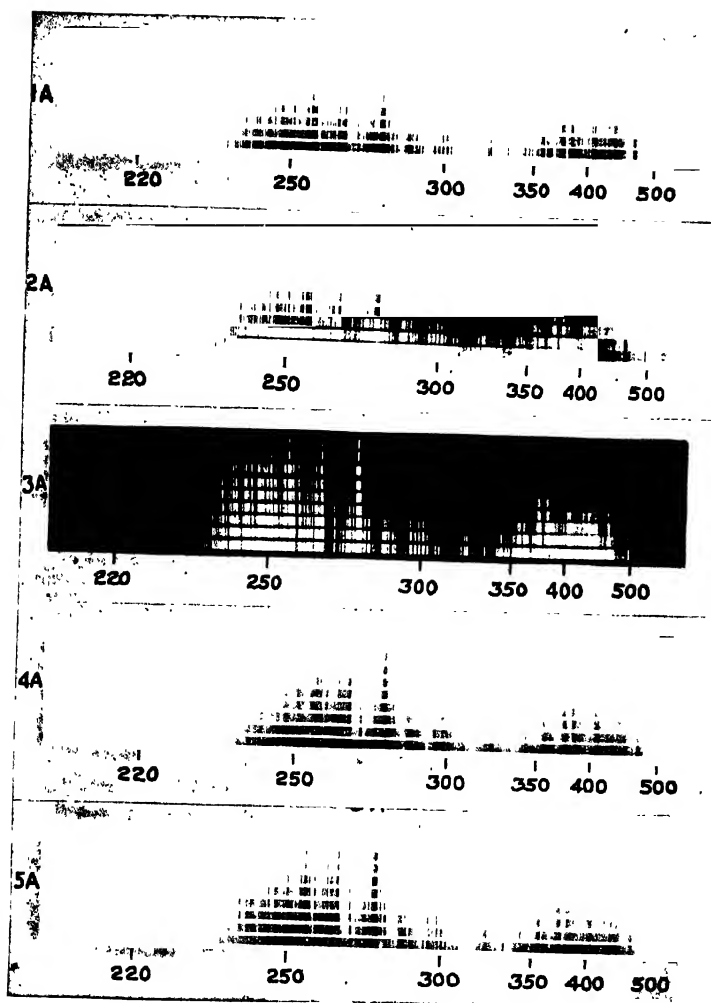


FIG. 4.

Series A: exposures were made in a Hilger ultra-violet spectrograph, and time of exposure varied so that each received an exposure inversely as the "speed" of the sample.

Series B: exposures were given through rotating sector cut in such a way that a logarithmic variation of the time of exposure was given.

The spectrograms show that the desensitizing did not produce *any detectable shift in the spectral sensitivity*. This confirmed the results of an earlier test on the blue-violet wedge spectra ⁸ of normal and desensi-

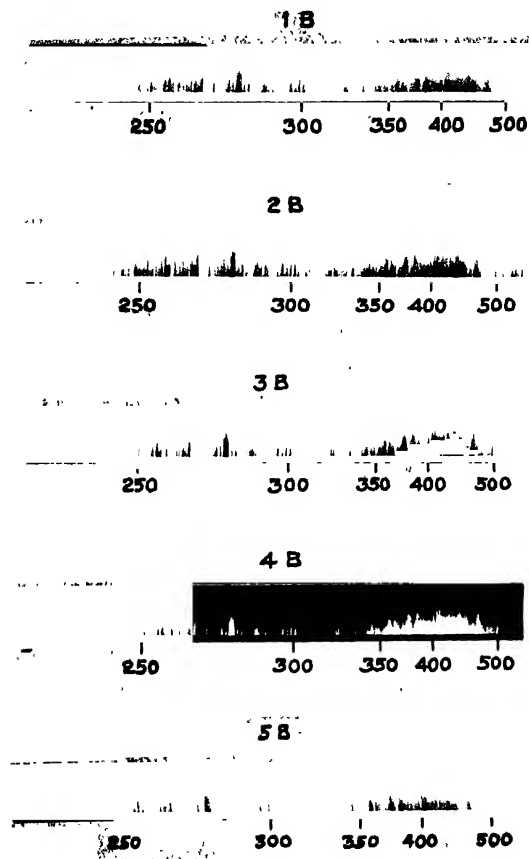


FIG. 5.

tized plates. In complete agreement with this conclusion are the results of Toy and Edgerton ⁹ on the relation between the light frequency and the number of developable centers in silver bromide grains. They have

⁸ Cf. Sheppard, Trivelli and Loveland, *J. Frankl. Inst.*, 200, 51 (1925).

⁹ *Phil. Mag.*, 48, 947 (1924).

shown that the number of centers produced at the three wave-lengths 4358, 4062, and 3650 Å.U. is proportional to the energy absorbed by silver bromide at these wave-lengths, the agreement being somewhat better if the energy absorbed is calculated in quanta.

The conclusion that the "sensitivity substance" is not an optical sensitizer appears to be thoroughly justified, and important consequences will be drawn from this. The next questions that arise concern the distribution of the "sensitivity substance" in the silver bromide grains, and the way it affects latent image formation. The work of Svedberg,¹⁰ Toy¹¹ and others has shown that the development of light affected silver bromide grains starts at isolated centers, which increase in size and

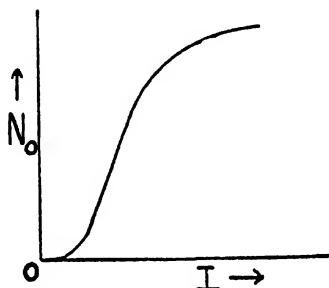


FIG. 6.

number as development proceeds, and also increase in number as exposure to light is increased. These "centers" appear to be scattered at random¹² over the surface of the grains, and the chance of a grain being developable depends upon its having at least one (developable) center. In explanation of these facts, Svedberg¹³ suggested a modified light quantum hypothesis of exposure, according to which a *developable center* is formed if a *sufficient number of light quanta hit the grain within a certain minimum area*. The light quantum hypothesis of Silberstein¹⁴ according to which it is sufficient for *one quantum* to hit the grain and be absorbed does not agree with the induction in the curve relating number of developable grains (of one size) to exposure¹⁵ (cf. Fig. 6). This curve should be of the same character as for α -particles, when it is found that every α -particle hitting a grain makes it developable¹⁶ (cf. Fig. 7).

Svedberg's quantum hypothesis is, however, inadequate, because it

¹⁰ *Phot. Journ.*, **62**, 186, 310 (1922).

¹¹ *Phil. Mag.*, **44**, 352 (1922).

¹² Svedberg, *loc. cit.*

¹³ *Loc. cit.*, *Phot. Journ.*, **62**, 310 (1922).

¹⁴ *Phil. Mag.*, **44**, 257 (1922).

¹⁵ Cf. Sheppard, Trivelli and Loveland, *loc. cit.*

¹⁶ Svedberg and Andersson, *Phot J.*, 1921, p. 331

takes no account of inherent grain sensitivity, and the existence of the "sensitivity substance" other than silver bromide, as demonstrated by chromic acid desensitization.

A theory proposed by Toy¹⁷ is based on this. It supposes that nuclei of this "sensitivity substance" are distributed at random over the surface of the grains. These nuclei were supposed to vary not only in *number* but in sensitivity to light, the sensitivities being distributed according to Maxwell's curve of molecular velocities. Further, this distribution was

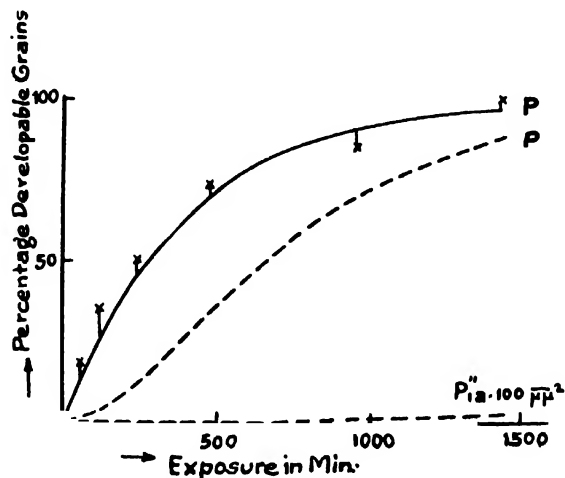


FIG. 7. (From Svedberg and Anderson.)

supposed to be shifted according to grain-size, so that a nucleus in a larger grain would be more sensitive than in a smaller one. This theory has been discussed and criticized elsewhere by the writer and his collaborators.¹⁸ It must suffice to point out that it is inadequate for the following reasons. First, the "nuclei" of "sensitivity substance" cannot themselves be photosensitive, as originally supposed by Toy. Because, if so, their absorption would determine the spectral sensitivity, and not that of the silver bromide, as actually found. Nor can they act as photocatalysts, accelerating the photochemical decomposition of the silver halide. For in this case: they might lessen the light energy required to reduce a silver ion to metallic silver, which means reduce the quantum absorbed, *i.e.* change the wave-length. But this is equivalent to optical sensitizing, which has already been ruled out. Again, they might increase the number of silver atoms reduced at the same

¹⁷ *Phil. Mag.*, 44, 852 (1922).

¹⁸ Sheppard, Trivelli and Loveland, *J. Frankl. Inst.* 200, 51 (1925).

wave-length. But this contradicts the photochemical equivalence principle, according to which the reduction of one silver atom results on the absorption of one quantum. Although Eggert and Noddack's¹⁹ results are not conclusive of this holding for silver bromide, they strongly suggest it, and certainly make it unlikely that more than one silver atom

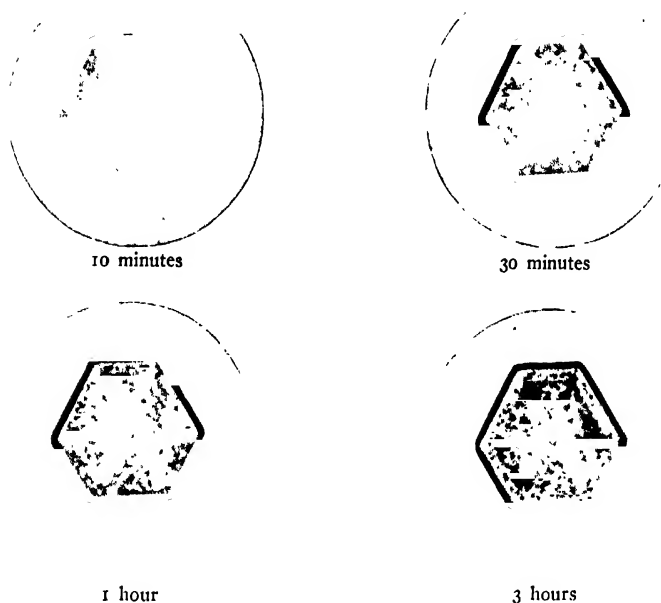


FIG. 8.

can be formed for one quantum absorbed. Toy and Edgerton's results²⁰ are also in agreement with this. Hence, it must be concluded that the "nuclei" of "sensitivity substance" are not photosensitive themselves, nor can they catalyze the photochemical decomposition of silver halide, in the sense of increasing the amount decomposed per energy absorbed. In what way then do they bring about their undoubted enormous increase of photographic sensitivity?

In recent papers²¹ the writer and his collaborators have proposed the following theory. Photographic sensitivity implies developability. We suppose that the nuclei of "sensitivity substance" do not affect the

¹⁹ Cf. also F. Weigert, *Zeitschr. f. Phys.*, 18, 282 (1923).

²⁰ *Phil. Mag.*, 48, 947 (1924).

²¹ Sheppard, Trivelli and Loveland, *Loc. cit.*

number of light quanta absorbed by the silver halide, but cause the photochemical change corresponding to this absorption to take place in their immediate neighborhood. In other words, they *orient* the photochemical change, so that there are concentrated about the nuclei *sufficient silver atoms within a minimum area* to give a developable center. The condition required by Svedberg is, therefore, achieved without the necessity of a certain number of quanta hitting the grain within a certain minimum area. In one of the papers referred to²² it is shown that this theory accounts for Silberstein's equation holding at high exposures, and for large grains. In another²³ it is shown that the conception of *orientation* of the photochemical effect in silver halide grains is supported by the microscopic observations on the visible decomposition. In larger, specially grown crystals, an *orientation* of the decomposition is clearly evident, as shown in the series illustrated. (Cf. Fig. 8.) Moreover, this orientation is in a large degree auto-catalyzed, in the sense that the photoproduct induces further decomposition to occur in contiguous silver halide. We have attempted to account for this orientation by the theory of ionic deformation, or of perturbed electronic orbits. Wherever there is any degree of disorientation in the crystal, the ions will be deformed. The energy absorbed anywhere in the crystal will tend to reduce this deformation, in general resulting in the transfer of an electron from a bromide ion to a silver ion.²⁴

In the papers referred to²⁵ it is shown that this conception is in good agreement with the statistical relation of sensitivity to grain size. The argument for it would, however, perhaps be considerably strengthened if the chemical nature of the "sensitivity substance" could be discovered, and if it were found to be a substance likely to have the requisite properties in regard to the silver halide lattice.

The Chemical Nature of the Sensitivity Substance

It was very early believed that in preparation of gelatino-silver halide emulsions some slight reduction of the silver salt took place, with the formation of either subhalide or colloid silver.²⁶ Lüppo-Cramer considered that his desensitizing experiments with chromic acid supported the hypothesis that the "sensitivity substance" or "Reifungskeime," as he termed the supposed nuclei of this, were colloid silver of high dispersity. He has in his numerous writings²⁷ consistently supported and developed this view of the matter. F. F. Renwick²⁸ in his notable

²² *J. Frankl. Inst.*, **300**, 51 (1925).

²³ *J. Physic. Chem.*, (to appear shortly).

²⁴ Cf. F. Weigert, *Zeitschr. f. Elektrochem.*, **23**, 367 (1917).

²⁵ *Loc. cit.*

²⁶ Cf. Trivelli and Sheppard, Monograph on Silver Bromide Grain of Photographic Emulsions. (Monographs on the Theory of Photography, No. 1, Eastman Kodak Company, 1921.)

²⁷ E.g., *Kolloidchemie und Photograph*, 2nd Edit.

²⁸ *J. Soc. Chem. Ind.*, **39**, 156T (1920).

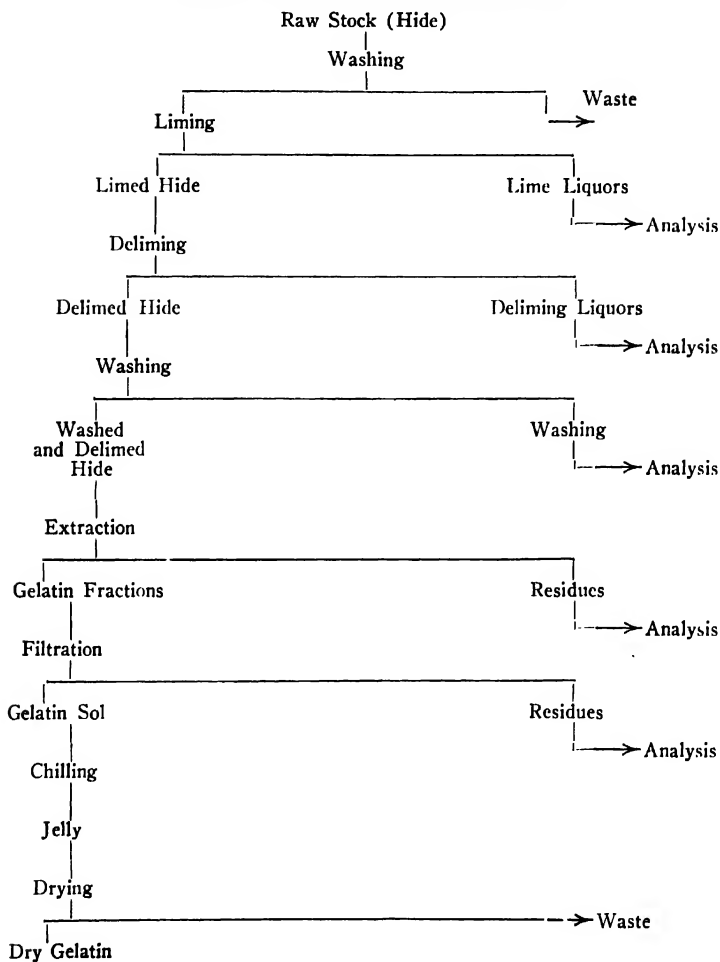
Hurter Memorial Lecture in 1920 also supported a colloid silver theory of sensitivity. On the other hand, W. Clark²⁹ and some of the British investigators have inclined to consider the sensitivity substance to be silver oxide or hydroxide, due to adsorption of OH ions. In this they have followed Fajans' and Frankenger's³⁰ work on the sensitizing of the visible decomposition.

Inasmuch as the effects described by Fajans and Frankenger are optical sensitizing effects, involving shift of the quantum $h\nu$ limit capable of reducing a silver ion, they are not applicable to the fundamental sensitizing effect under discussion. A series of investigations, carried out over a number of years at the Eastman Kodak Works in Rochester, N. Y., have led to the discovery of the real nature of the "sensitivity substance," the mode of its production, and the essential reactions in the photographic sensitizing of the silver halide grain. The publication of patents covering the technical and industrial applications of these discoveries makes it now possible to briefly describe the principal results, which were obtained by the coöperation of the manufacturing departments with the Research Laboratory. It has already been noted that different gelatins can give quite different photographic sensitivities with what is essentially, so far as silver halide characteristics go, the same emulsion. A fundamental advance which made possible the isolation and identification of the "sensitivity substance" was made by Mr. R. F. Punnett, of the Eastman Kodak Emulsion Department, when he succeeded in making from a very "active" gelatin an extract which could be used to sensitize other relatively inert gelatins. That is, this extract could be added to emulsions which were otherwise of low sensitivity, and greatly increase both their speed and density giving power. In conjunction with the Emulsion Department the writer made a prolonged investigation on the active principle contained in this extract. The story is too long to tell in full at this time. Analysis of the extract gave certain leads which were not at first successful but later proved of value. A lengthy series of substances were tested by adding aqueous or aqueous-alcoholic solutions at different concentrations to a standard test emulsion made up with a relatively inert gelatin. Hydrolytic and oxidative break-down products of gelatin, including a series of amino-acids, were tried without success. Various peptones and globulins, the derivatives of hemoglobin, purin extracts and the whole series of purin bases, as creatin, creatinin, xanthin, hypoxanthin, guanidin, were tested, also without effect. The writer, therefore, decided to follow up analytically the whole process of manufacture of gelatin, examining both the intermediate and the waste or by-products. The following chart shows the stages which had to be followed:

²⁹ Cf. W. Clark, *Brit. Journ. Phot.*, Dec. 14, 1923.

³⁰ *Zeitschr. Elektrochem.*, 28, 499 (1922); *Zeitschr. physik. Chem.*, 105, 255, 273, 329 (1923).

STUDY OF GELATIN MANUFACTURE

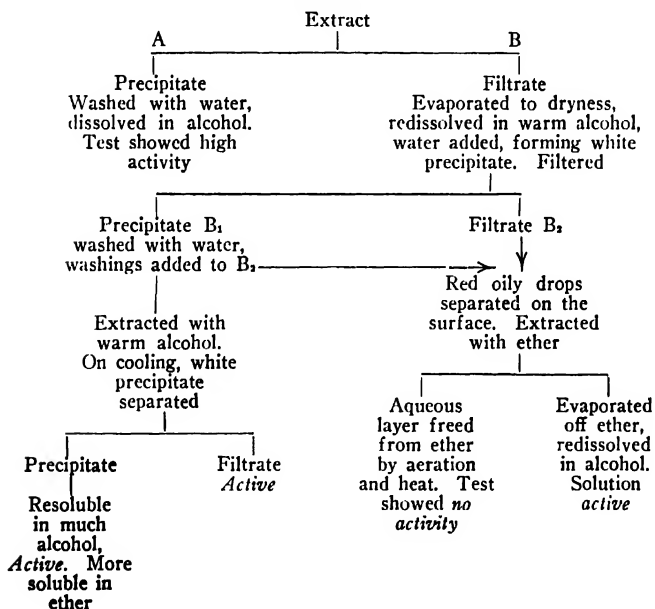


A fortunate circumstance enabled us to considerably shorten the investigation. A gelatin was investigated in the laboratory which gave excessive fog. It was found that extraction of this with acid greatly reduced the fogging propensity, while the acid extract after dilution and neutralization was found to have sensitizing properties. It was decided, therefore, to examine the acid deliming liquid which is used to neutralize

the excess of lime remaining after washing the limed hides. A quantity of limed hide stock was obtained and carefully delimed with 1 per cent HCl. The deliming and wash liquors were collected and concentrated *in vacuo* in a tin lined evaporator. On testing with the standard emulsion addition of 100 cc. to 1 gallon emulsion increased the speed from 15 to 24 Chapman Jones, the density (γ_{∞}) also being greatly increased.

On heating this extract to boiling, to find if the substance was heat stable, a coagulum was precipitated. The supernatant liquid was tested and found *inactive*. The coagulum was extracted with 95% alcohol, concentrated *in vacuo*, and slightly diluted with water. On testing it increased the speed from 15 to 21 C. J., and also increased γ_{∞} . A reduction in the amount of extract added lessened the fog and γ_{∞} but left the sensitivity unaffected.

TABLE III



Deliming liquors obtained from the Plant subjected to the same treatment gave confirmatory results. As the vacuum concentration of large quantities of these liquors is a tedious matter, it was decided to try another method of extracting the active principle. The fact that it was thrown down with the albuminous coagulum produced on boiling suggested that other hydrous precipitates might carry it down. A

separation by precipitating *hydrous alumina* in the deliming liquor was tried and found effective. This was confirmed several times on a small scale and then tried out on a large scale. About 4000 gallons of deliming liquor were treated in two large tanks. These were provided with steam coils for heating, and lined with fine meshed cloth to filter off the liquid and collect the precipitate. Ordinary potash alum was added, and hydrous alumina precipitated by caustic soda, the alkalinity not being allowed to exceed $\text{pH} = 8$, this being determined colorimetrically. The precipitate after settling overnight was collected and dried at about 70°C . This precipitate, or pulp, on extraction with ethyl alcohol, gave a very active extract.

TABLE IV

cc. of Extract in 50 cc. Alcohol	Speed		Gamma			
	C. J.	H. & D.	γ_s	γ_n	γ_v	Fog
0.0	15	63	.28	.40	.61	.01
0.5	17	93	.31	.54	1.81	.01
1.0	18	—	—	—	—	—
3.0	20	—	—	—	—	—
5.0	20 +	445	.66	1.08	2.74	.01
8.0	19	—	—	—	—	—
10.0	19	405	.60	1.18	—	.06
20.0	16	167	.78	1.21	2.50	.20

This procedure gave a relatively large amount of the "gelatin-x" in a form such that it could be concentrated in alcohol, and as at the same time a source of the crude material existed, it was hoped that isolation and identification might now be possible.

The following procedure enabled a new step forward to be taken. Several hundred grains of the dried aluminous pulp were ground to about 100 mesh, and extracted with alcohol; the extract was brownish-red and on concentrating to a fifth of its volume *in vacuo*, a *white* precipitate separated. This was filtered off, and chart (Table III) shows the principal details of the examination of this solid and the residual liquid.

These experiments showed that the "gelatin-x" could be separated as a solid body, which is somewhat soluble in alcohol but more soluble in ether, and as found later, in ligroin (petroleum ether). To avoid the fogging effects of ether on photographic materials, ligroin, tested itself for absence of fogging or sensitizing action, was now used for making extracts. It was found that good yields of the "gelatin-x" could be obtained from the dry aluminous pulp by extracting with ligroin, vaporating off the ligroin and redissolving in alcohol. Experiments with different amounts of the active body added to the standard emul-

sion now showed the important fact that, for the given emulsion and conditions, the *sensitizing effect reached a maximum* for a certain quantity and then decreased as the amount of active material was increased further. This is brought out in Table IV on p. 90.

The last of these showed an appearance of reversal in the lowest tones, and the latitude was very poor.

Decay or Change of Sensitivity Substance

At first it was found possible to extract the aluminous precipitate several times in repetition and secure considerable yields of the active principle. On storage of the finely ground dry pulp it was found, however, that the yield of active principle diminished with keeping. It could not be ascertained at the time whether this was due to volatilization, oxidation or some other change; the actual cause will be noted later. The original unground pulp did not lose activity so rapidly.

Nature of the Active Extract

On evaporation to dryness of the extract from the deliming solutions, a reddish colored waxy substance was obtained. When heated *in vacuo* a distillate or sublimate was obtained condensing to a mass of white acicular crystals, themselves rather soft and waxy. An alcoholic extract was made of this condensate, and found to be active.

Further examination, melting point determination, etc., showed that this condensate consisted chiefly of *cholesterol*. On the other hand, pure cholesterol⁸¹ gave us quite inactive solutions. It had, therefore, to be concluded that either the sensitivity substance was carried over as a soluble impurity with the sterol or we had to do with a substituted sterol differing from ordinary cholesterol. This was by no means an unlikely possibility, in view of the still imperfectly elucidated chemistry of the sterols.⁸²

Corresponding to this close association with sterols, it was found that not too drastic saponification with alcoholic caustic alkalis allowed saponifiable fats and oils to be removed without destroying the active principle. From the nature of this so far ascertained, it was thought worth searching for new sources of raw material and primarily in vegetable foodstuffs. The seed of plants seemed the most hopeful and quantities of different seeds were ground and extracted with ligroin. The ligroin was distilled off as usual and alcoholic solutions of the residues made and tested. Some of the results are shown in Table V.

It was found possible to remove the grease trouble by a preliminary saponification with alkali, then extraction with ligroin. In these extracts we again have a fraction consisting chiefly of sterols—so-called phyto-

⁸¹ Purified by the Synthetic Organic Chemicals Department, Eastman Kodak Company.

⁸² Cf. Windhaus, *Ber.*, 52, 170 (1919).

TABLE V

Material	Speed Increase C. J.	Density Increase	Fog	Remarks
Wheat flour	4	Good	Nil	Grease spots
White lima beans.....	5 +	"	"	" "
White haricot beans.....	5 +	"	"	" "
Split dry green peas.....	7 +	"	"	" "
Split dry yellow peas.....	5 +	"	"	" "
Whole rice	5 +	"	"	" "

sterols. Sublimation (or distillation) *in vacuo* again gave waxy crystals, having melting points around 139° C. but not perfectly sharp. An attempt was now made to separate the active principle from the sterol or sterols by the digitonin method. It was found, however, that much sensitizing material was carried down, perhaps by occlusion, with the digitonin steride. On recrystallizing the sterols from alcohol, it was found that the sensitivity substance distributed itself between the ethyl alcohol and the sterol, which is quite in agreement with the solubility relations found. This, also, with other indications, tended to confirm the view that the substance X is only dissolved in the sterols, and not chemically combined, and has, therefore, an independent origin. A further attack on the problem of separating "gelatin-X" from the sterols was planned. It was estimated that the active principle formed about 5 to 10 per cent of the sterol extract, with 90 to 80 per cent pure sterol present. At this time, however, it was noticed on heating the emulsions to which the very active seed extracts had been added that a garlic-like odor appeared. A quantity of mustard seed (*Sinapis nigra*) was now obtained, macerated in water, stood overnight and steam distilled. The distillate dissolved in alcohol was tested at various concentrations, and found to have many times as much active substance as any previous material.

Identification of Gelatin-X

These results clearly pointed to the sensitizer being either *allyl isothiocyanate* or *allyl sulfide*. A number of pure allyl compounds were obtained and tested in alcoholic solution.

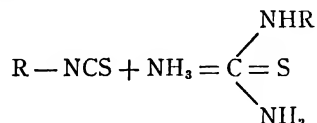
TABLE VI

Substance	Formula	Result
Allyl isothiocyanate	C_3H_5NCS	Very active
Allyl alcohol	C_3H_5OH	Inactive
Allyl amine	$C_3H_5NH_2$	Inactive
Allyl iodide	C_3H_5I	Inactive
Allyl sulfide	$(C_3H_5)_2S$	Inactive

Hence, the allyl group as such had nothing to do with it, *activity* deriving from the *isothiocyanate* radicle. This was confirmed with other *isothiocyanates*, as *phenyl*, *ethyl*, etc. The *normal* thiocyanates, *e.g.*, C_2H_5SCN had little or no activity.

The Chemistry of Photographic Sensitizing

It is well known that the isothiocyanates (thiocarbimides) are readily converted by heating with ammonia or amines to thiocarbamides, *e.g.*



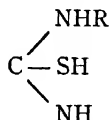
Tests were now made with the thiocarbamides (or thioureas) and, as anticipated, these were found to be very active.

TABLE VII

Substance	Formula	Speed Increase C. J.	Density	Fog
Thiourea	$\begin{array}{c} \text{NH}_2 \\ \diagup \\ \text{C} : \text{S} \\ \diagdown \\ \text{NH}_2 \end{array}$	+ 5	High	Considerable
Allyl thiourea	$\begin{array}{c} \text{NHC}_2\text{H}_5 \\ \diagup \\ \text{C} : \text{S} \\ \diagdown \\ \text{NH}_2 \end{array}$	+ 10	"	Slight
Allyl diethylthiourea...	$\begin{array}{c} \text{NHC}_2\text{H}_5 \\ \diagup \\ \text{C} : \text{S} \\ \diagdown \\ \text{N}(\text{C}_2\text{H}_5)_2 \end{array}$	+ 4	"	Nil
Thiosemicarbazide	$\begin{array}{c} \text{NH} \cdot \text{NH}_2 \\ \diagup \\ \text{C} = \text{S} \\ \diagdown \\ \text{NH}_2 \end{array}$	+ 7	"	Considerable

These results showed the grouping $\begin{array}{c} \text{N} \text{-----} \\ \diagup \\ \text{C} = \text{S} \\ \diagdown \\ \text{N} \text{-----} \end{array}$ to be of funda-

mental importance for sensitizing. It was found that when thioureas were converted and fixed in derivatives of the *pseudo*-form



that sensitizing was not effected.

Corresponding with this, tests showed that organic sulfur contained in mercaptans as $-\text{SH}$, sulfides $>\text{S}$, disulfides

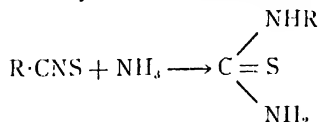


and in rings was inactive. In general, doubly bonded $=\text{S}$, as also $=\text{Se}$ and $=\text{Te}$ gave active bodies. Further, the most active sensitizing bodies tend to combine with silver halide, having in fact solvent properties or forming complexes.

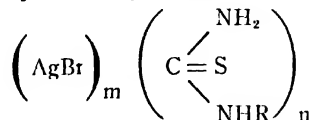
The "Sensitivity Substance"

The sensitizing body in photographic gelatin is primarily *allyl isothiocyanate*, which becomes partly converted to *allyl thiocarbamide*. The former is nearly insoluble in water, the latter fairly soluble. This explains certain curious and puzzling behaviors and changes both with gelatins, and with the materials investigated. For photographic sensitizing the following are the fundamental reactions:

i Conversion of isothiocyanate to thiocarbamide

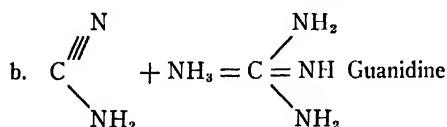
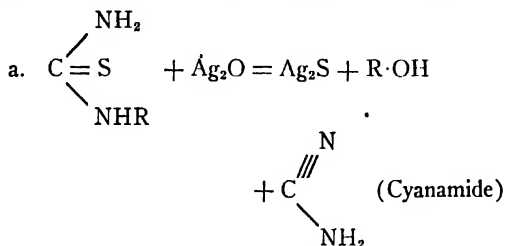


ii Combination of thiocarbamide or analogue with silver halide. This may be provisionally written

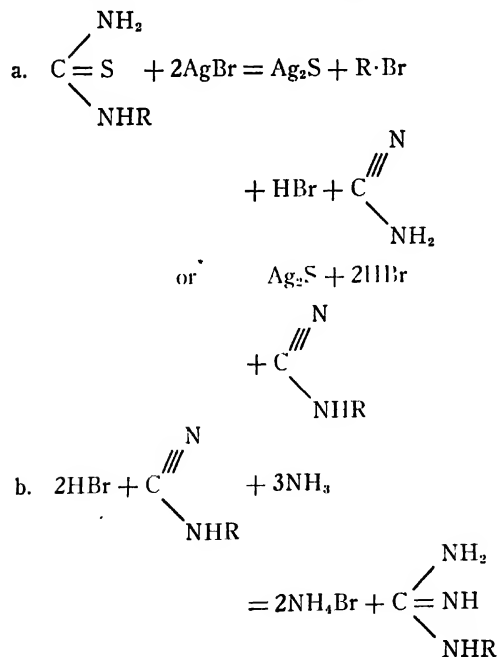


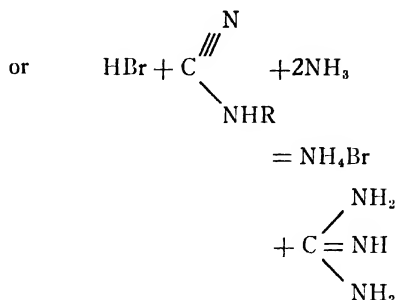
where m and n are simple integers

- iii Internal decomposition of these complexes, with formation of *silver sulfide*. In alkaline solutions silver salts react with thio-carbamides, etc., according to the general equations



In the case of silver bromide the reactions may be written :





ammonia being necessary to take up the HBr and secondarily perhaps to convert the cyanamide to guanidine.

The "sensitivity substance" discussed in the first part of this paper is, therefore, *silver sulfide* Ag_2S , which can be replaced by Ag_2Se or Ag_2Te with equal or greater effect. This substance is consistent with the theory developed for the function of the "sensitivity nuclei."

Silver sulfide is known to be capable of acting as nucleus in silver reductions. Further, its presence in the silver halide lattice would correspond to a sphere of disorientation (and ionic deformation) of the silver halide about it. It has been pointed out by G. von Hevesy³³ that the smaller the ratio of electrolytic conductivity just above and below the melting point, the easier the disorientation of the atoms in a (heteropolar) crystal lattice. For example, with the halides, the following table shows how this condition increases up to silver iodide:

TABLE VIII

Substance	Ratio of Conductances
AgI	0.9
AgBr	5.0
AgCl	30.0
TI	100.0
$\text{TI} \cdot \text{Br}$	130.0
$\text{TI} \cdot \text{Cl}$	160.0
NaCl	3000.0

This disorientation tendency is further related to the electron affinity of the ions forming the crystal. "The smaller the work necessary to convert the ions forming the crystal into the neutral state, the greater the disorientation tendency of the crystal." This principle is, moreover, reversible, and is fully in harmony with the theory of the sensitizing function proposed. Considering the silver halides, we have

³³ *Zeitschr. physik. Chem.*, 101, 337 (1922).

TABLE IX

Substance	Energy on Conversion to Neutral State in kg. cal.	Conductance Ratio
AgI	328—59 = 269	0.9
AgBr	328—67 = 261	5.0
AgCl	328—96 = 232	34.0

Silver iodide not only shows the greatest disorientation tendency, but lowest electron affinity. But further, as v. Hevesy points out, *sulfur* has an electron affinity of only 45 kg. cal., and solid Ag_2S shows an even greater conductivity than solid AgI, being the best known electrolytic conductor.³⁴

It appears probable that the well known importance of silver iodide in the gelatino-silver bromide emulsions is definitely related to the considerations advanced. More than a certain amount of silver iodide (*ca.* 5 per cent) is detrimental, because of the reduction of developability, but up to this amount its formation influences the structure of the crystal in the sense of increasing the disorientation. This effect is still further enhanced by forming nuclei of Ag_2S in the lattice; in the neighborhood of these nuclei the disorientation—and consequent deformation—of the silver halide ions will be much greater, and it may well be that any radiation absorbed by the crystal is effective, according to the photochemical equivalence principle, in reducing the silver ions about these nuclei. It must be remembered that these nuclei of Ag_2S are themselves contributing to the reducibility and developability. They only have to add around one of them a sufficient increment of silver atoms to ensure developability of the grain. If they are themselves increased, in sensitizing, above a certain size, they can lead, as the writer and his colleagues have already pointed out, to spontaneous developability or "latent fog."³⁵

It will be seen from this that the "latent image" must actually consist of colloid silver on nuclei of silver sulfide. Desensitizing is primarily due to the destruction of these silver sulfide nuclei. The residual sensitivity is a consequence of residual centers of disorientation, in some degree due to traces of silver sulfide too *protected* by silver halides to be destroyed by the oxidizer, *e.g.*, chromic acid. The results given and the theory proposed do not exclude some degree of participation of colloid silver in the sensitivity promotion, although it is probably quite secondary. It apparently becomes of first order importance only as the second order optical sensitizing effects, (small shifts in effective $h\nu$ limits in consequence of Stark effects with deformed ions) grow in magnitude,

³⁴ Cf. Tubandt, Eggert and Schibbe, *Zcit. anorg. Chem.*, 117, 22 (1921). In the β -form below its transformation point (170°) it is partly a metallic, partly electrolytic conductor, above, purely electrolytic.

³⁵ Wightman, Trivelli and Sheppard, *Phot. Journ.*, March, 1925.

when we get the panchromatizing effect of colloid silver discovered by Lüppo-Cramer.⁸⁶ It is also possible that the photoelectric sensitivity of Ag_2S contributes in some measure to its action, but rather indirectly by induction of electronic perturbations in the contiguous ions of the silver halide than directly.

A word is perhaps necessary on the amounts of the sensitizing mustard oil in photographic gelatins. By methods which I cannot delay to describe it has been found that this is of the order of 1 part in 300,000. The fact that such small amounts are operative, larger quantities than a certain limit (which varies with the type of emulsion) producing fog, together with the difficulties of detection and estimation in such an excess of a material like gelatin, no doubt explains why it has remained so long a mystery.

The present results have important bearings on both the theory of optical sensitizing and dye desensitizing, but these cannot be discussed here.

In conclusion it is my pleasant duty to express my thanks to colleagues in both the Works and the Laboratory for coöperation on many points, and particularly to Mr. H. Hudson for his careful and loyal assistance in the analytical operations. In subsequent papers fuller details and developments of the work will be published conjointly with them.

Summary

1. The nature of the photographic sensitivity of silver halides is discussed. A theory is proposed according to which nuclei of foreign material act as centers of ionic deformation in the silver halide lattice, so that the photochemical decomposition is oriented to occur about them rather than be dispersed over the crystal.

2. It is shown that the "foreign material" or "sensitivity substance" is derived from the gelatin.

3. The sensitizing property of photographic gelatins has been traced to the presence of *thiocarbinides* (organic isothiocyanates) and *thiocarbamides*, probably allyl mustard oil derived from food stuffs.

4. Reaction of the *thiocarbamides* with silver halides forms nuclei of silver sulfide, which is the "sensitivity promoting substance" or foreign material in the silver halide grains. *Silver selenide* and *silver telluride* can function equivalently.

5. It is shown that the properties of silver sulfide are in harmony with the theory of sensitivity advanced.

Research Laboratory of the Eastman Kodak Company (Communication No. 241),

Rochester, N. Y.

⁸⁶ Cf. *Kolloidchemie und Photog.*, 2nd Edit.

CATALYSIS BY METALLIZED SILICA GEL

L. H. REYERSON AND KIRK THOMAS

The properties of silica gel should make it an excellent carrier for metal catalysts. Accordingly investigations were started with the object of depositing finely divided metal upon the disperse surface of silica gel. As a result of some experimental work upon charcoals it was felt that hydrogen adsorbed by silica gel might reduce to the metallic state the ions of the metals which are below it in the electrochemical series. Experiments were finally successful and deposits of copper, silver, gold, platinum and palladium were obtained upon the disperse surface of silica gel by the reducing action of adsorbed hydrogen.

Briefly the method of procedure, as described by Latshaw and Reyerson,¹ was as follows: A sample of silica gel, prepared by Patrick's method, was heated to about 250° and evacuated at this temperature for about two hours. While still evacuated it was cooled to about -20° in a salt-ice bath and purified electrolytic hydrogen admitted. After adsorption equilibrium had been reached solutions of salts of the metals to be deposited were added. Tenth molar solutions were usually used. The process of reduction was not rapid but after several hours it had usually completed itself and the remaining solution could be drained from the gel. The metallized gel was then washed and dried. The deposits were usually dull to a lustrous black except in the case of gold when they were of a yellowish character. Platinous and palladious salts had to be used to obtain the best deposits of platinum and palladium. In the case of copper, reduction was usually incomplete and a further reduction with hydrogen was necessary.

The metallized gels as prepared in this manner still retained the porous character of silica gel. Crushing of the silica gel granules revealed that the metal deposit was general throughout the gel. In fact, microscopic examination definitely showed a complete covering of the disperse surface of the gel by metal. Fresh surfaces produced by fracturing the granules did not differ from the original surface. It was apparent from these results that a very thin metal film was coating the enormous surface of the silica gel. Because of its method of formation this film might even approach molecular dimensions. These metallized gels therefore would present a maximum surface of contact for gas adsorption or catalytic activity.

¹ Latshaw and Reyerson, *J. Amer. Chem. Soc.*, **47**, 610 (1925).

Experimental work on the catalytic activity of the metallized gels was therefore begun. The reaction between ethylene and hydrogen has long been used as an indication of the catalytic activity of a particular catalyst in reduction reactions. It was therefore chosen as the reaction to be first studied in connection with these catalysts. Equal volumes of ethylene and hydrogen were mixed and passed through the catalyst. About five grams of catalyst was used in each case and this was placed in a U-tube having an average cross section of about 1 square centimeter. The gas train was constructed so that the tube containing the catalyst and the aspirator bottle containing the fresh mixed gases could be kept at the same temperature. The dried ethylene-hydrogen mixtures were passed through the catalyst rapidly for several minutes to drive out any gases present in the catalyst. The flow of gas was then stopped and the catalyst allowed to adsorb the gases. When equilibrium was established the mixed gases were passed through the catalyst at the rate of 400 cc. per minute. Samples of the gas were taken after passing the catalyst and analyzed as follows.

The sample of the gas to be analyzed was carefully measured in a gas burette. It was then passed into a Hempel pipette filled with bromine water where any unchanged ethylene was removed. After treatment to remove any bromine from the gas by potassium hydroxide solution, its volume was redetermined. This gave the volume of unconverted ethylene by difference. Exactly half of the remaining gas was mixed with excess oxygen and burned over mercury. The products of combustion were then measured and passed into a pipette filled with potassium hydroxide. The loss in volume in this treatment represented the volume of ethane produced by the catalytic action. The percentage of ethylene converted was given by

$$100 \times \frac{\text{volume of ethane}}{\text{volume of ethylene} + \text{volume of ethane}}.$$

The results of a series of experiments are given in the following table:

Metal-gel Catalyst	Temperature	Number of Experiments	Average Conversion in Per Cent
Palladium	95°	3	98.9
Palladium	65°	3	84.4
Palladium	23°	4	91.4
Palladium	0°	6	93.5
Platinum	23°	3	71.8
Platinum	0°	4	60.9
Copper	23°	4	4.2

In the case of the gold and silver coated gels no conversion was obtained and this was true of the silica gel itself. The ethylene used

was commercial ethylene showing 95% absorption in bromine water. The electrolytic hydrogen analyzed 97% hydrogen.

The above experiments indicate marked catalytic activity even at zero degrees. Time-space yields have not been completed as yet but the above experiments were carried out under the same working conditions so that the results should show the comparative activity of the catalysts in simple reductions.

Because of the interesting results obtained in the hydrogenation of ethylene a series of qualitative experiments were undertaken, with the object of determining the type of reactions that these metallized gels might catalyze. Several attempts were made to reduce carbon monoxide and in one or two instances slight traces of formaldehyde were obtained when water gas was passed over the palladium catalyst at moderate temperatures. This may be due to the presence of enough oxygen to oxidize a trace of methyl alcohol that might be formed. This work has just been started and a detailed study of carbon monoxide reduction is planned.

A nickel gel catalyst was prepared by a slight modification of above mentioned method. The gel was treated as indicated and the liquid drained from it. The complete reduction of the nickel salt was then made by passing hydrogen over the gel at about 300°. This catalyst showed remarkable activity in ethylene reduction. An interesting point was noted in this connection. Often no reduction of ethylene could be detected at room temperature or lower. If however the catalyst were heated to 80° reduction was found to be nearly complete. It was then possible to cool the catalyst to room temperature, while the reacting gases were still passing through the catalyst, with no apparent change in the activity of the catalyst. This can be accounted for because of local heating caused by the reaction. Furthermore after more than one hour of operation the percentage of ethylene converted was if anything higher than at the start. The percentage conversion usually ranged from 90 to 99%.

This nickel catalyst was then used in the hydrogenation of phenol. Hydrogen was bubbled through heated phenol and this mixture was passed through a glass tube filled with the nickel catalyst. The tube was electrically heated and a constant temperature was maintained in the tube. With a large excess of hydrogen, more than 200% of the theoretical requirement, excellent production of cyclohexanol was possible when the catalyst was heated to 180°. It was found however that the activity of the catalyst diminished after several hours of operation. If the catalyst was then exposed to oxygen or the air and reduced once more with hydrogen its activity was found to be restored for another period. The average conversion to cyclohexanol in the experiments as carried out was from 50 to 60%. When an attempt was made to hydrogenate aniline, ammonia was liberated. The largest single fraction had a boiling

point which showed that the product of highest yield was probably phenyl-amido-cyclohexane.

The metal gel catalysts were also found to be active in oxidation reactions. If the platinum gel catalyst is placed in a U-tube, immersed in an ice water bath, and a mixture of hydrogen and oxygen passed through it, the catalyst will suddenly glow and oxidize the hydrogen completely. In fact only the first of the catalyst coming in contact with the gases will glow at all.

Experiments on the oxidation of ethylene have been carried out with the platinum gel catalyst. If the temperature of the catalyst be kept sufficiently low the ethylene will be incompletely oxidized. In several instances formaldehyde was detected as one of the products. It was impossible to identify the other products. A large number of experiments were also tried with methane oxygen mixtures. As yet none of these experiments has shown partial oxidation of methane. There was either no oxidation or else complete oxidation.

University of Minnesota,
Minneapolis, Minn.

COLLOIDAL WATER AND ICE

By HOWARD T. BARNES, D.Sc., F.R.S.

It appears to be a matter now of common knowledge that water is a highly associated liquid. Evidence is at hand from all of the physical properties of that liquid to prove that long before the freezing point is reached there is produced in increasing quantity molecular aggregates which have the same general characteristics as ice. In other words as I pointed out many years ago from a study of the variation of the specific heat of water with temperature, water contains dissolved ice molecules, and the concentration of this solution of ice increases right down to the freezing point, where saturation occurs, and any further cooling results in the appearance of the solid form.

There have been a great many papers written on the association of water, and attempts made to find the molecular complex of the ice aggregate. To Bragg we owe a debt of gratitude for giving us the crystal structure of ice from purely theoretical reasoning, and the picture he gives us is of surprising interest. It is unfortunate that the actual measurements by X-ray analysis do not accord quite with the theory, and do not in fact agree with each other, but considering the difficulty of knowing how the ice, which has been used in the measurements, is formed the agreement is extraordinary. When one realizes that water is highly colloidal, and that the first ice that forms from solution grows to a considerable size before taking on any sort of crystal shape we can see how the manner of producing the ice is of importance for X-ray study.

It is my intention here to discuss the many natural phenomena which are caused by finely divided ice and fog particles in water and air and to point out where direct observation has shown the existence of particles which have grown from associated molecules in every respect similar to true colloidal particles.

Color of Water and Ice

Some years ago I pointed out that the color of iceberg ice, which is so vivid in its beautiful blue tone, is probably due to the influence of

large group molecules. I tried to connect this fact with the presence of the ice molecule in the water by drawing attention to the wonderful color of pure sea water or glacier water free from dust, which showed the same wonderful blue. Recently Prof. Ramen in his address before the British Association at Toronto endeavored to bring forward evidence to prove molecular scattering as the cause of the color. It was pointed out in criticism of his contention that the Tyndall effect in ice was probably due to dust and that it was impossible to get ice or water free from it. The color of ice is a very good indication of its manner of formation and to any one used to comparing various samples it is easy to distinguish between samples produced under conditions prohibiting the presence of dust or foreign matter in its structure, and ice that has such imbedded therein. There is no question from careful conductivity tests that ice, formed on the underside of a thick sheet growing over flowing water, is as pure as it is possible to get anything in nature. Samples of such ice are absolutely sterile even from polluted water and every trace of foreign matter is eliminated by the slow freezing. Probably iceberg ice is the same although it is formed quite differently, and has quite a different structure. Iceberg ice shows no crystal structure and appears to be what one would expect from its formation under high pressure a mass of more or less circular grains as though the nuclear structure of the snow and frost flowers that compose it have slowly grown during the lapse of time. Therefore I believe that glacier ice and iceberg ice must possess the deepest and most beautiful colors.

The color of water is of great interest for here we have an example of so many different hues from impurities that it is difficult to know when we get it pure. Glacial streams and lakes are always a most wonderful blue and when cold are deep in shade. The water of the St. Lawrence river comes from the great lake system which is such a gigantic settling basin for the silt and mud that its water is so clear as to be noticeable wherever it can be seen. It is a wonderful sight in winter to watch the varying shades as the temperature changes. To one used to the river during the ice period it is possible to tell with a fair degree of accuracy when the temperature is at the freezing point, when the water is slightly supercooled and when it is slightly above 32° F. The whole color and general appearance changes. I believe this to be due to the ice molecules in solution which are at the point where they become visible first as minute microscopic globules, and afterwards as round discs or plates which increase in size, eventually giving the water the appearance of being loaded with sand. But this fact will be discussed presently. What I want to emphasize now is the remarkable similarity of a mass of water and a corresponding mass of ice just at the freezing

point. I am not speaking of ice as we ordinarily think of it, for, to most people, ice is ice. I am thinking of a mass of congealed water, or water which has flocculated, and in which the particles have grown to large dimensions, and have reached the crystalloid stage. To me ice, freshly formed, is nothing but a great clot, which gradually undergoes structural alterations. The slowness of formation of ordinary river or lake ice makes it naturally large and crystalline, but ice that has formed rapidly from water that is only slightly supercooled is of a very fine colloidal structure.

Frazil Ice

A very beautiful example of flocculation is afforded by the appearance of what we call in Canada Frazil ice. This form gives at times unlimited trouble to Power Operation in that it accumulates at the



FIG. 1.—Microscopic photograph of Frazil ice freshly formed showing small discs with no crystal shape. Magnification 2 diameters.

intake of the racks, or forms great hanging dams beneath the surface covering of the forebay. It packs in and obstructs the flow of the water like the slimy precipitate so often met with in chemical analysis. The manner of the formation of frazil is quite simple. It occurs as a filmy sort of curtain with streamers in the water of a river which is flowing too swiftly for surface ice to form. The temperature of the water is always slightly below the freezing point when the ice appears. Below the freezing point the water is supersaturated with ice molecules and the flocculation is due to this fact. The ice, as it first

forms, is so fine that it cannot be seen, but it soon agglomerates into spongy masses of loose texture, which catch onto objects immersed in the water and quickly builds together into an immense mass. The minute particles composing the mass are as already pointed out disc shaped and in consequence these orientate themselves like the scales of a fish and become impervious to the flow of the water. In a very few hours the largest Power house will be shut down. The remedy is very simple for the smallest application of heat, sufficient to offset the slight cooling of the water, causes it to disappear as by magic. As long as the temperature does not fall below 32° F. no frazil can form, but the smallest drop below that point results in its appearance. Indeed the first indication of the ice comes often before any drop is to be noticed on a delicate thermometer. To one experienced in ice work the condition of the water just as it is ready to produce the frazil can be seen from its general color and appearance which seems to show that the particles are growing to such a size as to influence the light effects in the water.

In Figure 1 which accompanies this text is shown a microscopic study of freshly formed frazil ice as it is taken from the St. Lawrence River on a cold morning in the winter before the sun has got high enough to influence the temperature of the water. The small well defined discs of ice can be readily seen which show no definite crystalline shape. Later in their development they begin to take on the appearance of snow crystals but not in running water where the small particles are rapidly disintegrated and kept of small irregular dimensions due to continual wearing by the water.

Anchor Ice

This form of ice appears on the bottom of the river wherever no cover protects the bottom from the excessive radiation which takes place on cold clear nights. Of all the ice on the upper St. Lawrence anchor ice is the most abundant and forms by far the greatest proportion of the ice flowing down. It forms on points or dark objects when the water is undercooled at night usually between 11 p. m. and 6 a. m. and often attains depths of 5 feet or more. Immediately on the advent of dawn when the scattered radiation glows in the eastern sky the anchor ice begins to rise and floats down stream. With the direct rays of the sun the ice rises completely and the bottom becomes quite clear until the following night when it grows again. Undoubtedly the deposition of ice results from the contact of the supercooled water loaded with the excess ice particles, and this takes place easily on sharp points and on surfaces that offer ready radiation during periods of excessive nocturnal cooling.

Mist and Fog

Recent work on the nature of mist and fog is based on the existence of a nucleus on which the larger molecular aggregates attach themselves. G. C. Simpson writes about this in a recent paper in the Scientific

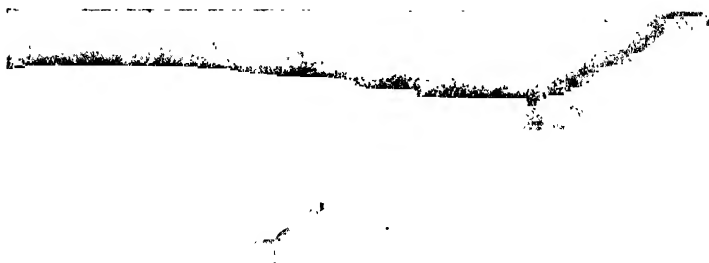


FIG. 2.—Photograph taken 9 A.M., January 28th, 1925, of black fog. St. Lawrence River, at Morrisburg, Ontario.



FIG. 3.—St. Lawrence River at Morrisburg, same afternoon of black fog. Open water surface clearly seen, between the bordage ice. The river never freezes over here owing to its swift current.

Monthly and it seems to me that colloidal particles form the nucleus.

Probably the most interesting form of fog, which has received little attention, is the black fog which occurs over the St. Lawrence River in

very cold weather. It is a phenomenon of fairly rare occurrence, but when it comes it is a very interesting sight. Mist and winter fog are quite common over the open water surface whenever the temperature drops to 11 degrees or less and is in nature like the highest clouds composed of fine ice particles. The black fog occurs only at extremely low temperature and appears to be a heavy precipitation of large semi-

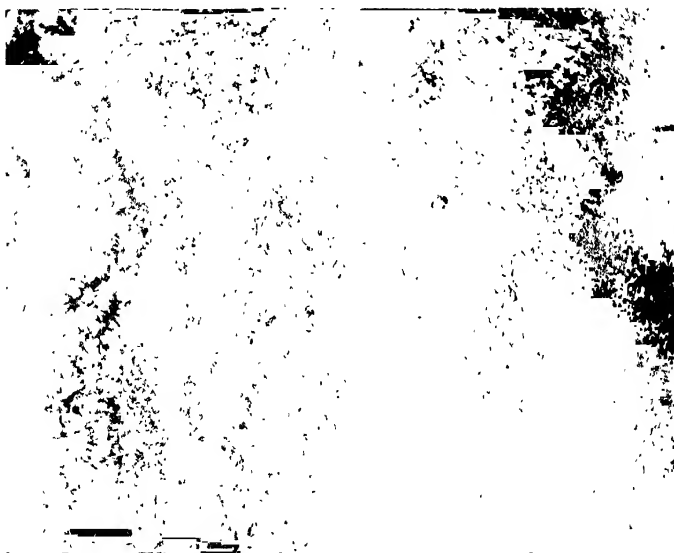


FIG. 4.—Window frost showing transition from fresh deposit of fine colloidal particles to well defined crystal forms. Taken at Cornwall, Ontario.

fluid globular particles which absorb completely the energy of the sunlight. It usually comes out of a clear atmosphere over the open surface of the river with sub-zero temperatures and is preceded by a white fog rising to heights of 500 feet or more in long streamers, a truly wonderful sight. With the rising of the sun the white fog subsides and changes rapidly from sulphur yellow like burning sawdust to deep opaque black at the bottom. This shuts out completely all view of the water, of the objects on the surface and of the opposite shore. In Figures 2 and 3 I show photographs taken last winter of one of the thickest black fogs ever seen. No. 2 is taken at 9 a. m. just after the sun was up and No. 3 at 4 p. m. with fog gone. On rowing out into this blanket the temperature was much higher than the surrounding air; in fact the fog felt

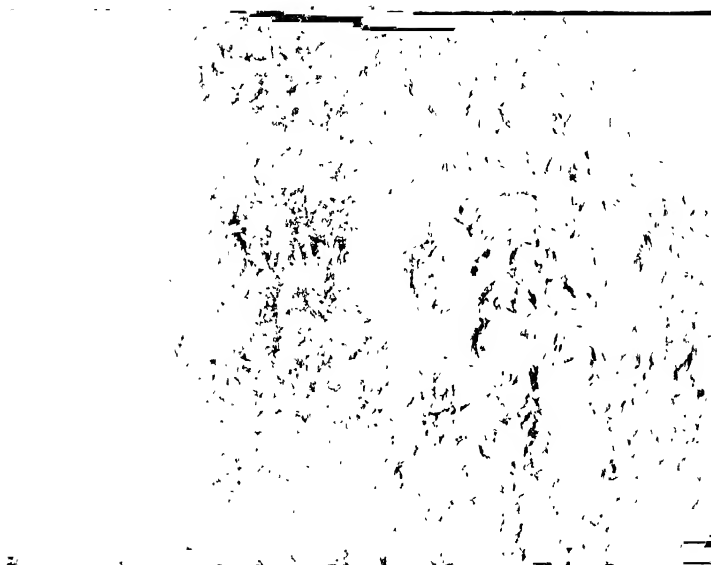


FIG. 5.—Window frost showing more advanced stage of crystal development.
Taken at Cornwall, Ontario.

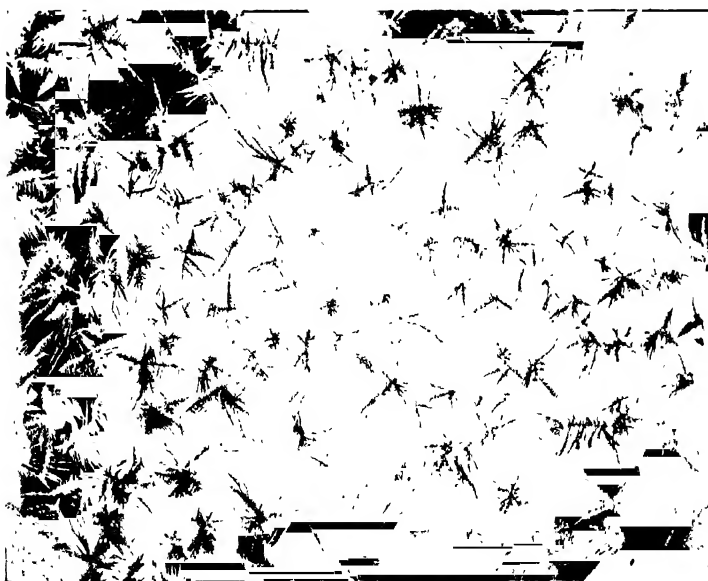


FIG. 6.—Window frost showing well advanced stage of crystal development.
Note the many four-pointed star shapes, indicative of the influence of
Sodium Chloride.

quite warm to the face. This blanket of fog is one of the most effective protections to the excessive loss of heat from the open water and much less ice is formed during these periods.

Ordinary white fog blows in towards shore and is seen to be formed of plates and minute particles of ice. This is deposited on grass and trees and gives rise to some very beautiful effects.



FIG. 7.—Window frost showing effect of uncleaned glass on the crystal form.
Morrisburg, Ontario.

Snow appears to have its origin from colloidal particles, to judge from the many beautiful pictures which W. A. Bentley has obtained. The central nucleus of his photographs is almost always a point or round figure without crystalline shape.

Frost patterns on glass offer an interesting study of colloidal influence on crystallization. In the preceding four pictures I show some of these patterns taken last winter at Morrisburg and Cornwall, Ontario.

In one of them we can see probably the influence of sodium chloride in the four prong star shape patterns. In one of the photographs we can see the gradual alteration from the simple colloidal particle deposited from the air to the accumulated crystal form with the peculiar ramifications caused by the influence of the glass itself and any impurity left on the glass.

Many of Bentley's pictures show that a spherulitic phase, probably composed of groups of colloidal crystals made spherical by surface tension, precedes the formation of regular crystals and even of dendrites. There are marked instances of this in plates 13, 15, and 17 in W. A. Bentley's collected papers in the *Monthly Weather Review* of 1907. My attention was directed to these by Mr. Jerome Alexander, to whom I am much indebted for focusing my attention on this branch of ice study. The existence of Halos is probably due to colloidal ice in the atmosphere. The gradual increase in the size of the molecular groups undoubtedly gives us all grades from the fine haze in the atmosphere to the white fogs and gradual alteration to the thick yellow and black fogs. It seems that there should be some careful measurements of these fogs in relation to the ideas which seem to help us explain them the best in the light of colloidal phenomena. The known existence of the "white death" or "pogonip" of the Blackfoot Indians indicates the existence of excessive hoar-frost.

Glass being a colloid influences crystal structure as we have seen in the many and varied patterns of window frost.

Fine colloidal particles appear to be responsible for the haze over the summer sea in which sodium chloride plays an important part. We are only just at the threshold of our knowledge of the molecular groups in water which on evaporation produce what we may term the colloidal form of water and which produce the nucleus for the ice crystals. So general is this influence on all chemical reaction and on crystal form that it has been completely overlooked in the past.

In this brief paper I have tried to point out a few of the outstanding phenomena connected with colloidal water and ice. In a future paper I shall show more in detail how important these molecular forms in water are on chemical activity.

Department of Physics, McGill University,
Montreal, Canada.

THE COLLOID CHEMISTRY OF RENNET COAGULATION¹

By L. S. PALMER AND G. A. RICHARDSON

The most common biochemical phenomena are often the most difficult to explain. As a result, widely divergent views are advanced, usually irreconcilable, but, as a rule, eminently satisfactory to their authors. The clotting of cow's milk by the addition of minute quantities of rennin is an example. The literature dealing with this phenomenon is extensive. Much of it is confusing. Many of the theories advanced to explain the process have been described as "weird." Some of them are indeed naïve.

In the present paper we shall attempt: first, to review the theories of rennet coagulation which have a more or less definite colloidal basis; second, to offer experiments which definitely refute the idea that colloidal protection is involved in the process; third, to present data bearing on the chemical differences between casein and paracasein which will help explain both the nature of the enzyme action and the ease of coagulation of paracasein; and, finally, to reconcile such of the definitely established facts as may be possible with the colloid-chemical laws with which they appear to be compatible.

Colloidal Theories of Rennin Action

It is not surprising that colloid enthusiasts have from time to time viewed the clotting of milk as a purely colloidal phenomenon. It has been known since the observations of Schübler² that the casein is not in true solution in cow's milk, but merely suspended as innumerable particles. The colloidal nature of the casein suspension has been verified by many investigators using dialysis and ultra-filtration methods. Kreidl and Neumann³ were the first to describe the appearance of the colloidal particles in the ultramicroscope. Alexander and Bullova⁴ made similar observations and Stübel⁵ has given us ultramicrophotographs of two stages of the rennet coagulation.

¹ Published with the approval of the Director as Paper No. 548, Journal Series, Minnesota Agricultural Experiment Station.

² Schübler, *Deut. Archiv. f. Physiol.*, 1819, No. 4; cited by Kreidl, A., and Neumann, A., *Archiv. f. d. Gesamt. Physiol.*, 123, 528 (1908).

³ Kreidl, A., and Neumann, A., *Archiv. f. d. Gesamt. Physiol.*, 123, 523-537 (1908).

⁴ Alexander, J., and Bullova, J. G. M., *Archiv. of Pediatrics*, 27, 18-25 (1910)

⁵ Stübel, H., *Archiv. f. d. Gesamt. Physiol.*, 156, 361-400 (1914).

The first purely colloidal theory of rennet coagulation was apparently advanced by Hammarsten⁶ who expressed the belief that rennin destroys something which holds a calcium caseinate-calcium phosphate complex in suspension. Hammarsten abandoned this view in favor of the chemical theory which has been the most widely accepted of any so far advanced.

Alexander⁷ was the first to express a theory similar to Hammarsten's early view, using modern colloid chemistry terminology. According to Alexander, the coagulation of milk by rennin is caused by a destruction of the colloidal protective properties of lactalbumin which holds the casein in suspension, "with the probable simultaneous production of coagulating substances," such as albumoses and peptones. Alexander was led to this theory by his ultramicroscopic studies^{4, 8} on milk and by his classification of the casein and lactalbumin of milk as irreversible and reversible colloids, respectively. Having adopted Zsigmondy's classification for these colloids, Alexander saw at once that the well-known protective effect of emulsoids (reversible colloids) for suspensoids (irreversible colloids), if applied to milk, would explain the apparently stable suspension of casein visible in the ultramicroscope on the grounds of the protective effect of the lactalbumin. On this basis it is obvious that the only possible explanation of rennin coagulation is that of a destruction of the protective effect of the lactalbumin.

In support of his theory Alexander has stated^{4, 8} that protective colloids like gelatin and gum arabic are capable of preventing the coagulation of casein by both acid and rennin. He has shown⁷ that lactalbumin will stabilize colloidal suspensions of AgCl and $\text{Ca}_3(\text{PO}_4)_2$ but that it no longer does so after digestion with pepsin.

Alexander has employed his theory to account for the failure of mother's milk to clot with rennin like cow's milk, it being well known that albumin equals or exceeds the casein in mother's milk, but is relatively low as compared to the casein in cow's milk.

Critical examination of Alexander's theory indicates that the assumption upon which the entire theory rests is open to question. This assumption is that the casein in milk is a suspensoid requiring a protective colloid for its stabilization. The ease with which stable dispersions of pure calcium caseinate can be secured without the use of protective colloids, and exhibiting identical chemical and physical properties of milk so far as coagulation with rennet and acid are concerned, cannot be reconciled with Alexander's assumption. This fact was apparently demonstrated first by Hammarsten and has been verified by many investigators as well as by us. Moreover, a calcium caseinate dispersion is itself a protective colloid of a high order, emulsifying fats,

⁶ Hammarsten, O., *Maly's Jahresberichte f. Tierchem.*, **4**, 174 (1874).

⁷ Alexander, J., *Proc. Eighth Inter. Cong. Applied Chem.*, **6**, 12-14 (1912).

⁸ Alexander, J., *J. Am. Chem. Soc.*, **32**, 680-689 (1910).

protecting gold sols, and exhibiting other properties of emulsoid sols. There are, in fact, scarcely any grounds for regarding the casein compound natural to cow's milk as an unstable, irreversible colloid. The only possible basis for such an assumption is the fact that ultramicroscopically calcium caseinate dispersions are identical in appearance with suspensoid sols. This point will be discussed later.

The statements of Alexander regarding the protective effect of added emulsoids on the acid and rennet coagulation of cow's milk are also open to question. We shall consider this point in some detail a little later. It will be sufficient to say at this point that Alexander's statement⁹ that "the addition of protective colloids to cow's milk stabilizes it and makes it behave like mother's milk when treated with acid or rennin," is based solely on his observations^{4, 8} as to the behavior of "very highly diluted" milk both macroscopically and in the ultramicroscope after the addition of gelatin or gum. The results of such a study are not to be regarded as an index of the behavior of undiluted cow's milk when treated with gelatin.

Schryver¹⁰ has advanced a "protection" theory of rennet coagulation somewhat similar to Alexander's. Schryver's view is that in ". . . milk, the materials necessary for clot formation pre-exist, but that aggregation is prevented by the adsorption of simpler molecules from the system," the function of rennin being to ". . . clear the surface of such substances (casein) of adsorbed bodies, and thus allow aggregation (clot formation) to take place."

In support of this hypothesis Schryver obtained data showing that solutions of calcium caseinate, containing added CaCl_2 in too great a concentration to give either a clot or a coagulation on warming, would give a clot on addition of rennin. It was also found that the concentration of CaCl_2 which would give coagulation of casein when added to calcium or sodium caseinate solutions failed to do so in the presence of an essentially protein-free milk serum,¹¹ Witte's peptone, and glycerine; whereas, when rennin was added, coagulation occurred.

Schryver's theory has been widely quoted and is regarded by Alexander as a confirmation of his protective colloid theory. However, Schryver himself was forced to conclude that "although many of the facts appear to support the hypothesis . . . the same can not be said to be definitely proved by the facts elicited in the study of milk clotting." Especially contradictory to his hypothesis was the fact that casein, which had been clotted by addition of CaCl_2 to milk, retained all its original properties when redissolved; whereas, casein, clotted by rennin, could not be clotted again by the enzyme.

The question of the replotting of rennet-clotted casein has been a

⁹ Alexander, J., "Colloid Chemistry," 2nd Edition, p. 130, 1924, D. Van Nostrand Co.

¹⁰ Schryver, S. B., *Proc. Roy. Soc. (Lond.) B.*, 88, 460-481 (1913).

¹¹ Filtrate after heat coagulation of whey from rennet-clotted milk.

matter of some dispute. Peters¹² claimed that solutions of paracasein in lime water coagulate again on the addition of rennet extract and that the process can be repeated as often as desired. Bang¹³ likewise has stated that under suitable conditions paracasein can be made to coagulate repeatedly with rennet. Hammarsten¹⁴ was the first to explain successfully that this apparent anomaly is due to the failure to exclude NaCl from the rennin solution, an observation which was verified by Laqueur¹⁵ and apparently overlooked by Bang.

Colloidal explanations of the milk clotting phenomena have by no means been confined to the destruction of a protector for the colloidal casein. Since the pioneer experiments of Hammarsten¹⁶ it has been accepted by the majority of students that there are at least two distinct stages in the rennet clotting. The first stage is the change of casein to paracasein by the rennin. The second stage is confined to the precipitation of the paracasein by the soluble calcium salts of the milk. According to Loevenhart¹⁷ the rennin also performs the function of making the calcium salts available for the coagulation stage of the process. This conclusion is based on the observation that there is normally a definite delay between the formation of paracasein and its coagulation and that this can be prevented by the addition of CaCl_2 at the end of the first stage of the reaction.

It is interesting to note that both stages in the rennet coagulation process have been explained on the basis of colloidal behavior, sometimes one and sometimes the other stage being emphasized as a colloidal phenomenon, and occasionally both stages being regarded as colloidal. It will be sufficient to review only the more significant of these theories.

Considering first the enzyme stage of the process, the most widely accepted views have been chemical, rather than colloidal. Two representative views will be mentioned. Hammarsten¹⁶ believed that rennin hydrolyzes a calcium caseinate-calcium phosphate complex into a calcium-calcium phosphate-rich paracaseinate and a calcium-poor whey-protein, while Bosworth¹⁸ believes that the hydrolysis is one similar to the hydrolysis of maltose, rennin producing two molecules of paracaseinate for each molecule of caseinate, whatever calcium was combined with the caseinate being divided between the two molecules of paracasein.

Of the colloidal views of the enzyme stage of the phenomenon,

¹² Peters, R., Preisgekrönte Schrift von der med. Facultät der Univ. Rostock. Rostock, 1894.

¹³ Bang, I., *Skand. Archiv. f. Physiol.*, 25, 105-144 (1911).

¹⁴ Hammarsten, O., *Zeit. f. Physiol. Chem.*, 22, 103-126 (1896).

¹⁵ Laqueur, E., *Biochem. Centralb.*, 4, 333-347 (1905-6). According to Laqueur the NaCl provides calcium for the coagulation through an exchange of bases with the calcium paracaseinate.

¹⁶ Hammarsten, O., *Nova Acta Regiae Soc. Sci. Upsaliensis in Memoriam Quattuor Saec. ab Univ. Upsaliensi Peractorum.*, 1877.

¹⁷ Loevenhart, A. S., *Zeit. f. physiol. Chem.*, 41, 177-205 (1904).

¹⁸ Bosworth, A. W., *J. Biol. Chem.*, 15, 231-236 (1913); *ibid.*, 19, 397 (1914).

Loevenhart¹⁷ concluded that rennin causes an association of casein particles into paracasein, a less highly dispersed colloid. Laqueur¹⁸ came to the opposite conclusion; namely, that paracasein is the more highly dispersed colloid because of the splitting off of the whey protein. Inichoff¹⁹ has recently come to a like conclusion, holding that the chemical cleavage described by Bosworth is in reality a colloidal peptization by the rennin, aided by H^+ and bivalent metallic cations. The conclusion is based on a slight increase in conductivity during the clotting process which Inichoff regards as due to an increase in the number of particles in suspension. According to the ultramicroscopic observations of Bleyer and Seidl²⁰ calcium paracaseinate particles are always smaller than calcium caseinate, and their Brownian movement is twice as rapid, thus supporting the views regarding the peptizing action of rennin. An especially interesting colloidal view of rennin action is that of Mellanby²¹ who regards it as changing the emulsoid, casein, into the suspensoid, paracasein. According to this view the rennin could be regarded as a denaturing enzyme so far as the hydrophilic properties of casein are concerned. As already stated, the ultramicroscope indicates that calcium caseinate solutions are already suspensoids. At least the aggregates of molecules are either very large, thus accounting for their very milky appearance in the pH range of milk; or their hydration is relatively low, which determines their visibility. In the latter case the rennin might be conceived as clearing the surfaces of the particles of adsorbed water. It would not be expected that a dehydrating action of this kind would be permanent, so that the hypothesis falls to the ground in the light of the evidence that paracasein dispersions are not clotted again by rennin.

Turning now to the coagulating stage of the phenomenon we find somewhat more unanimity of opinion regarding the colloidal nature of the process. Hammarsten¹⁴ held that the colloidal calcium-calcium phosphate-paracasein complex is precipitated or clotted by soluble calcium salts or NaCl in proper concentration, depending upon whether the complex is calcium paracaseinate (flaky coagulation) or calcium paracaseinate-calcium phosphate (clot formation). Little attention has been paid to this detail by most investigators who have been satisfied to secure a coagulation without bothering to report its nature.

Fuld²² has been quoted²³ as regarding the whole process of rennet coagulation as a special case of the mutual suspension and precipitation of colloidal substances. As a matter of fact, Fuld's statement was made solely in connection with the second stage of the clotting phe-

¹⁷ Inichoff, G. S., *Biochem. Z.*, **131**, 97-108 (1922).

¹⁸ Bleyer, B., and Seidl, R., *Kolloid Zeitschr.*, **30**, 117-118 (1922).

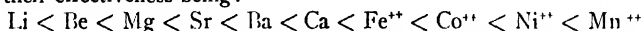
¹⁹ Mellanby, J., *Proc. Physiol. Soc. J. Physiol.*, **54**, cxvi (1921).

²⁰ Fuld, E., *Beiträge Z. chem. Physiol. u. Path.*, **2**, 169-200 (1902).

²¹ Loevenhart, A. S., *loc. cit.*; Kastle, J. H., and Roberts, N., *U. S. Hyg. Lab. Bull.* **56**, 352, 1909.

nomenon. It referred to the probability that calcium paracaseinate is not precipitated by calcium salts until a sufficient amount of paracasein is formed to overcome the stabilizing influence of the calcium caseinate, and that the first coagulum of paracaseinate carries down with it the residual unchanged caseinate.

Loevenhart¹⁷ investigated the relation of various cations to the precipitation of the colloidal casein and paracasein compounds as they exist in milk. In his study none of the monovalent cations coagulated either caseinate or paracaseinate. The paracaseinate, however, was coagulated with much greater ease than the caseinate by divalent cations, the order of their effectiveness being:



This was likewise true of all the heavy metals including ferric ions. The anions were without influence when comparing chlorides, sulfates, and nitrates.

Bang¹⁸ regarded the coagulation stage of the rennet phenomenon as analogous to the salting out of proteins by neutral salts. In his opinion a sharp distinction between casein and paracasein is not warranted, although he admitted that calcium paracaseinate is coagulated by much less CaCl_2 than a corresponding calcium caseinate. The explanation, according to Bang, lies in the fact that casein is merely the first member of a group of compounds with increasing affinity for calcium, the compound with the greatest affinity being coagulated by the least amount of calcium.

Van Slyke and Bosworth²⁴ have presented some quantitative data on the relative affinity of casein and paracasein for calcium. According to their results, casein and paracasein show no difference in calcium combining capacity when saturated with base, one gram of each combining with 90×10^{-5} gram equivalents of calcium. The so-called acid caseinates and paracaseinates, however, showed marked differences, one gram of mono- and dicalcium caseinate combining with 11×10^{-5} and 22×10^{-5} gram equivalents of calcium, respectively; the corresponding mono- and dicalcium paracaseinates combined with 22×10^{-5} and 45×10^{-5} gram equivalents of calcium. Strontium and barium paracaseinates of like composition are reported. Van Slyke and Winter²⁵ have prepared a similar set of magnesium caseinates.

Before concluding this preliminary discussion brief mention should be made of the fact that rennin itself exhibits the properties of a true colloidal enzyme. These have been summarized by Fuld²⁶ who pointed out that rennin forms colloidal solutions in water which withstand dialysis, and is readily adsorbed by indifferent proteins and animal char-

²⁴ Van Slyke, L. L., and Bosworth, A. W., *J. Biol. Chem.*, **14**, 203-231 (1914).

²⁵ Van Slyke, L. L., and Winter, O. B., *J. Biol. Chem.*, **17**, 287-297 (1914).

²⁶ Fuld, E., *Ergebnisse der Physiol.*, **1**, 468-504 (1902).

coal. Its albumin-like properties are shown by the fact that it is salted out by nearly complete saturation with $(\text{NH}_4)_2\text{SO}_4$. Vigorous shaking of rennet extracts inactivates them through adsorption in the surface layers of the foam.²⁷

EXPERIMENTAL

Relation of Protective Colloids to Rennin Behavior

In fairness to Alexander's repeated assertions^{4, 7, 8, 9} that protective colloids prevent the coagulation of milk by rennin, it should be stated that we have found several instances in the literature in which rennet coagulation has been retarded by colloidal substances but no cases in which it was prevented. For example, Bang¹³ added recrystallized serum albumin and ovalbumin to dilute solutions of calcium caseinate and found a retardation which he believed to be due to the adsorption of the rennin by the added proteins. Using a 1.2 per cent calcium caseinate solution he found also that the addition of 0.3 per cent lactalbumin retarded the coagulation in comparison with the lactalbumin-free solution. This retardation was regarded as due to the competition of the paracasein and lactalbumin for the calcium chloride present. Doyon²⁸ and Tadokoro and Sato²⁹ have found that sodium nucleate delays the coagulation time with rennin, but there is no indication from their results that the coagulation is prevented. The Japanese investigators report no definite effect of gelatin although the well-known effect on red gold sol is noted.

It is important to point out that in none of the cases cited was any attempt made to determine the effect of the colloidal solution on the pII of the milk or the casein solutions employed. The relation of this factor to the rapidity of coagulation with rennin has been known since the observations of van Dam³⁰ which were confirmed by Allemann³¹ and many others.

Effect of Rennin on Protective Properties of Lactalbumin.—We first repeated the observations of Alexander⁷ on the protective effects of lactalbumin on a silver chloride sol both before and after treatment of the albumin with rennin.

The albumin was prepared from supercentrifuged skim milk by diluting with an equal volume of saturated, neutral (to litmus), $(\text{NH}_4)_2\text{SO}_4$ solution and then adding NaCl to saturation. The clear filtrate was treated with 5 per cent H_2SO_4 solution until it had a pII of

²⁷ Schmidt-Nielsen, Signe and Sigval, *Zeit. f. physiol. Chem.*, **68**, 317-43 (1910).

²⁸ Doyon, M., *Compt. rend. Soc. Biol.*, **83**, 918-19 (1920).

²⁹ Tadokoro, T., and Sato, S., *J. Biochem. (Japan)*, **1**, 433-43 (1922).

³⁰ Dam, W. van., *Zeit. f. physiol. Chem.*, **58**, 295-330 (1908).

³¹ Allemann, O., *Biochem. Zeit.*, **45**, 846-858 (1912).

4.57 as shown by potentiometric measurement. The precipitated lactalbumin was dissolved in 300 cc. of distilled water and dialyzed against running distilled water for 96 hours when it no longer gave a sulfate test with BaCl_2 but still contained a trace of chlorides sufficient to give a AgCl sol with AgNO_3 . The crystal clear, colorless, slightly viscous solution contained 0.86 per cent lactalbumin on the basis of its nitrogen content.

The rennin used for the test was a fresh rennet extract (Marshall's) which had been dialyzed free from chlorides and filtered. Two cc. of 1 per cent solution of the dialyzed extract gave a typical gel in 18 minutes when added to 100 cc. of cow's milk at 40°C .

The lactalbumin and dialyzed rennet showed only scattered particles in the ultramicroscope. Table I shows the results of the experiment.

The experiment shows that rennin exerts no detrimental effect on the protective action of lactalbumin during the period and at concentrations in which milk is clotted. Even after 18 hours standing at room temperature the silver sol with the treated albumin was entirely stable.

TABLE I
EFFECT OF RENNIN-TREATED LACTALBUMIN ON AgCl SOL

Condition of Lactalbumin	Volume Lactalbumin, cc.	Volume AgNO_3 (2.9%), cc.	Temp. of Sol., $^\circ \text{C}$.	Appearance in Ultramicroscope
Untreated	2	.05	20	Single particles in active Brownian movement.
Treated with rennin *.....	2	.05	20	
Untreated	2	.05	35	Clusters of particles in slow motion.
Treated with rennin *.....	2	.05	35	

* 10 cc. lactalbumin + 0.2 cc. 1 per cent dialyzed rennin held at 40°C . for 20 minutes and cooled to temperature at which AgNO_3 was added.

Effect of Gelatin on Rennin Clots.—Two different samples of gelatin were used to determine whether this highly protective colloid affects the rate of clotting or character of the clot when added to cow's milk. Separator skim milk was used throughout. The experiments were planned to illustrate (1) the effect of the pH of the gelatin solution, (2) the effect of the concentration of gelatin, and (3) the effect of long standing in contact with gelatin. All tests were run in duplicate, using 100 cc. volumes. The clotting time was taken as the time of first visible coagulation as noted when a film of the milk was allowed to flow down the inside of the gently tilted beaker in which the test was conducted. The temperature used was 40°C . throughout, controlled by immersing the beakers in a water bath. Freshly diluted undialyzed rennet extracts

were used. Hydrogen ion concentrations were determined by the potentiometer using the Bailey electrode. The gelatin solutions were with one exception 1.25 per cent solutions freshly prepared a few hours before use by allowing the powdered gelatin to swell for 15-20 minutes in the required amount of water and then warming to 50° C. until dispersed. In the experiments in which the maximum possible protection was exerted, the dispersed solution was kept at 50° C. for 6 hours, cooled to room temperature, and allowed to stand 12 hours before use. Tables II and III summarize the data obtained.

The data in Tables II and III show clearly that gelatin exerts no retarding effect on rennet coagulation. On the contrary, the rate of clotting is accelerated even after the milk and gelatin have stood in contact for a period of 10 hours at 15° C. In addition, it is shown that when milk is diluted with an equal volume of gelatin solution of such strength that the concentration of protective colloids in the milk exceeds that of the casein (as is the case in mother's milk), a clot results with rennin at an accelerated rate, whereas the dilution of milk with water gives only a fine precipitate on long standing at clotting temperatures.

TABLE II

EFFECT OF ACIDITY AND CONCENTRATION OF GELATIN SOLUTION ON RATE OF CLOTTING AND CHARACTER OF CLOT OF COW'S MILK

Gelatin No.	Casein, Per Cent	Lactalbumin and Globulin, Per Cent	Gelatin, Per Cent	Ratio of Casein to Protectors	pH	Time, Minutes	Character of Clot
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(a) Using 1 cc. 10 per cent rennet extract per 100 cc.

I *	2.00	0.52	0.0	1:0.26	6.79	2.66	Firm
I	1.25	0.32	0.0	1:0.26	6.76	4.25	Very soft
I	2.00	0.52	0.5	1:0.51	6.69	2.25	Firm
I	1.25	0.32	1.25	1:1.25	6.65	2.12	Firm
II †	2.50	0.65	0.0	1:0.26	6.62	2.00	Firm
II	2.00	0.52	0.5	1:0.51	6.52	1.50	Firm
II	1.25	0.32	1.25	1:1.25	6.28	0.50	Firm

(b) Using 1 cc. 1 per cent rennet extract per 100 cc.

I *	2.50	0.65	0.0	1:0.26	6.65	23.25	Firm
I	1.25	0.32	0.0	1:0.26	6.81	80.00	Fine ppt.
I	1.25	0.32	1.25	1:1.25	6.64	20.25	Moderate
II †	2.50	0.65	0.0	1:0.26	6.62	18.00	Firm
II	2.00	0.52	0.5	1:0.51	6.52	13.50	Firm
II	1.25	0.32	0.0	1:0.26	6.75	60.00	Fine ppt.
II	1.25	0.32	1.25	1:1.25	6.28	5.25	Firm

* Gold number of gelatin = 0.004; pH of 1 per cent solution = 6.12.

† Gold number of gelatin = 0.023; pH of 1 per cent solution = 3.60.

TABLE III

EFFECT OF CONCENTRATION AND TIME OF CONTACT WITH GELATIN ON RATE OF CLOTTING AND CHARACTER OF CLOT

Volume Milk, cc.	Volume Water, cc.	Volume Gelatin Sol., cc.	Ratio of Casein to Protectors	Time Allowed for Equilibrium	Time of Clotting, Minutes
100	0	0	1:0.26	15 minutes	23.25
100	0	0	1:0.26	10 hours	22.50
50	50	0	1:0.26	15 minutes	No clot in 80 min.
50	50	0	1:0.26	10 hours	No clot in 60 min.
50	0	50 *	1:1.25	15 minutes	20.25
50	0	50 *	1:1.25	10 hours	20.50

* A 2.5 per cent gelatin solution, gold number 0.004, pH of 1 per cent solution 6.12.
 Note: 1 cc. of per cent rennet extract per 100 cc. was used.

Effect of Lactalbumin on Clotting of Calcium Caseinate with Rennin.—The relation of lactalbumin to rennet clotting was studied by means of solutions of calcium caseinate and lactalbumin. The casein was prepared by the improved method described by Van Slyke³² and yielded a snow white powder, readily dispersed by alkali. Two solutions of lactalbumin were employed, one of which has already been described. This preparation was concentrated by means of a fan until it contained 1.51 per cent protein before it was used in these tests. The second solution of lactalbumin was prepared by removing the casein from supercentrifuged skim milk with HCl, neutralizing the filtrate to pH = 6.5 with Ca(OH)₂, running the cloudy solution through the supercentrifuge to remove the precipitated phosphates, freezing the solution, decanting the concentrated unfrozen protein-sugar-salt solution, and dialyzing against running distilled water until only a trace of sugar remained. The precipitated globulin was filtered off and the filtrate concentrated by fan until it contained 1.6 per cent protein. Both preparations of lactalbumin were neutralized to pH of 6.5-6.7 before use.

The calcium caseinate dispersion was prepared by dissolving 15 grams of the pure casein in sufficient lime water to give a pH of 8.0, neutralizing to pH of 6.5 with 1 per cent H₃PO₄ solution, adding 2.5 c.c. of 0.1 M CaCl₂, and diluting to 300 c.c. volume. The pH of this artificial milk containing 5 per cent casein was 6.41. Table IV shows the results of the albumin-casein study.

The data in Table IV show that lactalbumin exerts no influence on the rennin coagulation of calcium caseinate. The slight retardation in the clotting of tests 3 and 4 represents merely a slight destruction of enzyme during the digestion with the lactalbumin. Especially interesting is the fact that a clot resulted in test 5 although the casein: albumin ratio was 1:1.28, similar to that in mother's milk which never clots. The

³² Van Slyke, L. L., *Proc. World's Dairy Congress*, 2, 1145-1151 (1923).

weaker gel obtained was due to the lower concentration of casein and calcium ions, the former being only one per cent, the latter being furnished solely by the calcium caseinate solution.

TABLE IV
EFFECT OF LACTALBUMIN ON CLOTING OF CALCIUM-CASEINATE BY RENNIN

Vol. Casein Sol., cc.	No. Albumin Sol.	Vol. Albumin Sol., cc.	Vol. Water Added, cc.	Vol. 1 Per Cent Renmet Ext., cc.	Time of Clotting (Minutes)	Remarks
10	I	0	10	0.1	9.5	Good clot, normal syneresis
10	I	10	0	0.1	9	Distinctly better clot
10	II *	10	0	0.1	12	Good clot, normal syneresis
10	II *	10	0	0.1	15	Good clot, normal syneresis
4	II	16	0	0.1	11	Clot weak, normal syneresis

* In these tests the rennin was allowed to act on the lactalbumin solution at 40° C. for 15 minutes before adding to the calcium caseinate.

The Behavior of Mother's Milk Towards Rennin.—It seems obvious to us that the failure of mother's milk to clot with rennin or to show even a coagulation in many cases must be sought on other grounds than that of protective colloids. In a recent note, one of us³³ pointed out that the failure of mother's milk to clot like cow's milk is to be explained on the grounds of its low concentration of casein and its high pH, Davidsohn³⁴ having reported the pH of mother's milk to be 6.97, and Clark³⁵ having reported a value of 7.22.

It appears that Szydlowski,³⁶ Meyer,³⁷ Fuld and Wohlgemuth,³⁸ Bienenfeld³⁹, and also Kreidl and Neumann³ have already shown that the addition of acid to mother's milk permits it to coagulate with rennin. We have secured a similar result by adding rennet to fat-free mother's milk which had stood until the pH had dropped to 5.6. There was no clot, however, but merely a precipitation of fine flakes.

Bosworth⁴⁰ has explained the failure of mother's milk to curdle with rennin on the grounds that not only are the casein and soluble calcium salts present in mother's milk in relatively small amounts but that the casein is probably present as potassium caseinate.

We have tested the effect of adding CaCl_2 to a sample of mother's milk (pH = 6.72) which gave no visible precipitate on long warming with rennin and found that a fine flocculent precipitate appeared with

³³ Palmer, L. S., *Ind. and Eng. Chem.*, **16**, 974 (1924).

³⁴ Davidsohn, H., *Zeit. f. Kinderheilk.*, **6**, 11 (1913).

³⁵ Clark, W. M., *J. Med. Res.*, **31**, 431-53 (1914-15).

³⁶ Szydlowski, Z., *Prager med. Wochenschr.*, **17**, 365 (1892).

³⁷ Meyer, L. F., *Berl. klin. Wochenschr.*, p. 1439, 1906.

³⁸ Fuld, E., and Wohlgemuth, J., *Biochem. Zeitschr.*, **5**, 118 (1907).

³⁹ Bienenfeld, B., *Biochem. Z.*, **7**, 262-287 (1907-8).

⁴⁰ Bosworth, A. W., *Am. Jour. Dis. Child.*, **22**, 193-201 (1913).

rennin after 35 minutes at 40° C. if sufficient CaCl_2 is added to increase the soluble calcium about 135 per cent. We have also found that mixtures of potassium caseinate and lactalbumin containing 1 per cent casein and 1.25 per cent lactalbumin at pH 6.36 give no coagulation with rennin. Potassium caseinate also inhibits the clotting of calcium caseinate unless the latter contains an abundance of soluble calcium salts as shown by the results in Table V. The calcium caseinate solution used was that previously described, containing 5 per cent casein with pH of 6.41. The potassium caseinate solution was prepared by dissolving 5 grams of pure isoelectric casein in 100 cc. of water containing 2.7 cc. 0.1 N KOH. The solution was opalescent and had a pH of 6.42.

Acid and Alkali Binding of Casein and Paracasein

In spite of the evidence of a number of the earlier investigators that rennin splits off a protease-like compound from casein, the investigations of Van Slyke and Hart,⁴¹ Van Slyke and Bosworth,²⁴ Geake,⁴² and Wright⁴³ have established that isoelectric casein and paracasein are chemically indistinguishable by elementary analysis, amino acid distribution (Hausmann numbers), or racemization methods. The outstanding chemical difference between the substances which has a bearing on their colloidal property of forming gels is indicated by the observations of Van Slyke and Bosworth²⁴ that paracasein combines with twice as much base as casein for the lower concentrations of alkali. Hoffman and Gortner⁴⁴ have shown that chemical binding of acids and alkalis by proteins occurs within the range of hydrogen ion concentration represented by pH of 2.5 to 10.5. Inasmuch as the rennet clotting phenomenon occurs within this range, it was believed that a careful comparison of the acid and alkali binding of casein and paracasein⁴⁵ would throw light on the difference in colloidal behavior of these substances as well as settle the question of the existence of the various alkali caseinates and paracaseinates described by Van Slyke and Bosworth.

The paracasein was prepared from skim milk which was warmed and passed through the Scharples supercentrifuge. The milk was next dialyzed free from soluble salts in collodion bags, diluted with an equal volume of water, and held at 30° C. in the presence of an excess of rennet extract for a half hour. After cooling to room temperature the paracasein was isolated and purified by the same improved method employed for the casein as described by Van Slyke.³² The final product

⁴¹ Van Slyke, L. L., and Hart, E. B., *Am. Chem. Jour.*, **33**, 461-496 (1905).

⁴² Geake, A., *Biochem. J.*, **8**, 80-87 (1914).

⁴³ Wright, N. C., *Biochem. J.*, **18**, 245-251 (1924).

⁴⁴ Hoffman, W. F., and Gortner, R. A., Colloid Symposium Monograph II, 209-868, 1925. Chemical Catalog Company.

⁴⁵ The data relative to acid and alkali binding of casein and paracasein are taken from the Thesis of G. A. Richardson for M.S. Degree, University of Minnesota, 1925.

TABLE V
EFFECT OF POTASSIUM CASEINATE ON CLOTTING OF CALCIUM CASEINATE
BY RENNIN

Volume Calcium Caseinate, cc.	Volume H ₂ O, cc.	Volume Potassium Caseinate, cc.	Volume 1 Per Cent Rennet Ext., cc.	Additional N/1 CaCl ₂ , cc.	Time Clot (Minutes)
10	10	0	0.1	0	9.75
10	10	0	0.1	0	10.00
10	0	10	0.1	0	No coag.
10	0	10	0.1	0	" "
10	0	10	0.1	0.2	" "
10	0	10	0.1	0.4	9.50

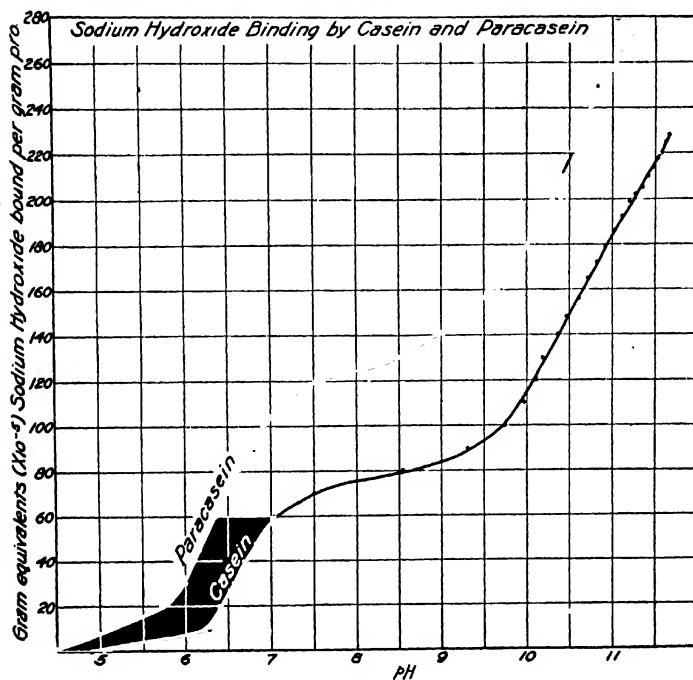


FIG. 1.

was a very fine, white powder, readily wetted by water and easily dispersed in alkalis.

In making the following series of determinations, we were fortunate in having at our disposal most of the apparatus used by Hoffman and

Gortner. The procedure followed was essentially the same as in their studies. A one per cent protein suspension was prepared by carefully wetting one gram of pure casein or paracasein with 100 cc. of distilled water in a Bovie vessel. Hydrogen was bubbled through the mixture using a modification of the Hildebrand type of bubbling electrode, which permitted the hydrogen to escape through the tube enclosing the electrode. Potentiometric measurements were made of the $[cH]$ on ad-

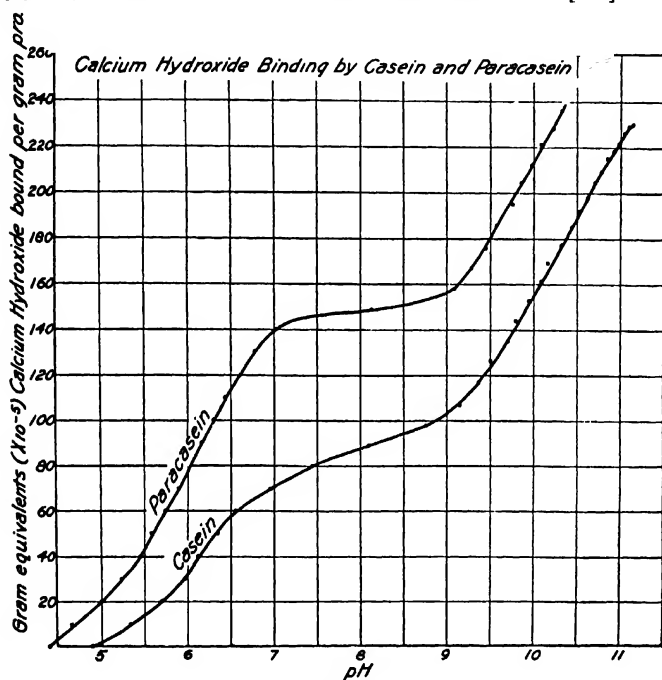


FIG. 2.

dition of 0.01 cc. increments of 1 N NaOH, 1 N viscogen ($Ca(OH)_2$ + sucrose), and standard HCl, respectively, 10 to 15 minutes being allowed for equilibrium. Enough titrations were made to draw an average curve. The Bailey electrode was used to check doubtful readings.

The normality of acid or alkali bound per gram of protein was calculated from the difference between the normality of acid or alkali alone and the normality of acid or alkali when protein was present. The normality of the NaOH and HCl solutions in terms of cOH and cH were taken from the data of Hoffman and Gortner. Similar values for pH and cOH of the $Ca(OH)_2$ were determined and are shown in Table VI.

Tables VII, VIII, IX, X, XI and XII present the data obtained and calculated from the potentiometric titration of casein and paracasein with NaOH, $\text{Ca}(\text{OH})_2$, and HCl. Figures 1, 2, and 3 show graphically the relation between casein and paracasein when the gram equivalents of alkali and acid bound are plotted as functions of the pH.

The acid and base binding data and their corresponding curves are not to be interpreted rigidly as showing the absolute binding capacity of either casein or paracasein at all concentrations or at all pH values. However, the conclusion of Van Slyke and Bosworth that casein and paracasein dissociate as polyvalent acids is not supported by the graphs. The same may be said for the conclusion of Cohn⁴⁶ that the titration

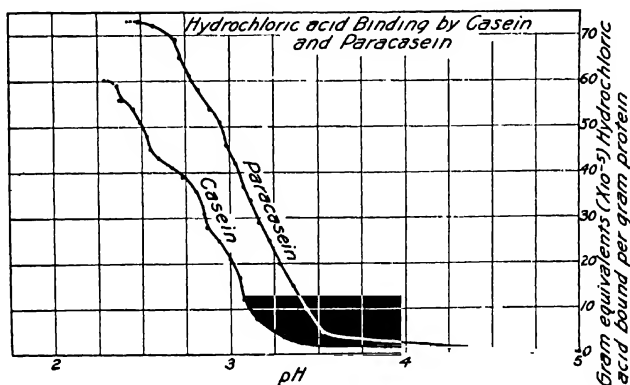


FIG. 3.

curve of casein with NaOH shows that casein dissociates "as an acid of at least two different strengths." Caseinates with the minimum amount of base bound to form a definite compound contain 11×10^{-5} gram equivalents of base per gram of protein, according to Van Slyke and Bosworth, and 49×10^{-5} gram equivalents of base, according to Cohn and Hendry.⁴⁷ These "compounds" are on the first buffer slope of our curves and are therefore not to be regarded as definite entities. Only a single calcium caseinate is indicated by our data, containing $80-90 \times 10^{-5}$ gram equivalents of base per gram of casein. The corresponding paracaseinate contains 150×10^{-5} gram equivalents of calcium per gram of protein. The base enters these compounds in the neighborhood of the neutralization of the second and third hydrogen of orthophosphoric acid by $\text{Ca}(\text{OH})_2$ as has been pointed out by Hoffman and

⁴⁶ Cohn, E. J., *Proc. Soc. Biol. Chemists, J. Biol. Chem.*, 52 ix-xi (1922).

⁴⁷ Cohn, E. J., and Hendry, J. L., *J. Gen. Physiol.*, 5, 521-554 (1923).

TABLE VI
HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF CALCIUM
HYDROXIDE AT 27° C.

Cc. N/1 Ca(OH) ₂ in 100 cc. Water	N	E.M.F.	pH	cOH
0.0	0.000	701.4	7.074	1.20×10^{-7}
0.1	0.001	882.9	10.141	1.40×10^{-6}
0.2	0.002	906.0	10.532	3.455×10^{-6}
0.4	0.004	928.0	10.904	7.955×10^{-6}
0.5	0.005	937.8	11.070	1.178×10^{-5}
0.7	0.007	939.6	11.100	1.383×10^{-5}
0.8	0.008	945.9	11.206	1.6285×10^{-5}
0.9	0.009	949.6	11.269	1.895
1.0	0.010	952.5	11.318	2.11
1.5	0.015	964.2	11.518	3.226
2.0	0.020	970.4	11.621	4.224
2.5	0.025	976.1	11.718	5.2855
3.0	0.030	981.4	11.807	6.464
3.5	0.035	986.4	11.892	7.853
4.0	0.040	990.4	11.959	8.238×10^{-5}
5.0	0.050	997.8	12.085	1.23×10^{-4}

Gortner.^{4a} It is possible, however, that a further refinement in technic may show the existence of a more acid compound in the region between the isoelectric point and the first buffer slope, although the calcium hydroxide curves make this doubtful. Nevertheless, the remarkable plastic properties of Van Slyke and Bosworth's "mono-calcium" caseinate and paracaseinate require a further study of this point.

Especially striking is the uniformly higher base and acid binding capacity of paracasein in comparison with casein, confirming in general the results of Van Slyke and Bosworth. Our results do not substantiate their conclusion that the base-saturated caseinates and paracaseinates contain the same amount of base and that there is a definite quantitative ratio of 1:2 between the base binding of "acid" caseinates and paracaseinates. Only at the pII of fresh milk is the ratio approximately 1:2, and at this pH the definite alkali caseinates and paracaseinates that are indicated by our results are still in the process of formation.

In spite of these disturbing features in connection with the comparative chemistry of casein and paracasein, the results do appear to have a definite bearing on the colloid chemistry of rennet coagulation. It is obvious that rennin, acting on an incompletely formed calcium caseinate in colloidal dispersion at the pII of milk, converts it into a much less completely formed calcium paracaseinate, the chemical binding capacity of which for both base and acid is permanently altered. The nature of the molecular rearrangement or surface change (since a substance, in colloidal dispersion is altered) causing the increase in binding is not

^{4a} Hoffman, W. F., and Gortner, R. A., *J. Phys. Chem.*, **29**, 769-781 (1925).

TABLE VII
ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT CASEIN SOLUTION
PLUS VARYING AMOUNTS OF SODIUM HYDROXIDE

Cc. N/1 NaOH	N	E.M.F.	pH	cOH	N ₁	n	Gm. Eqt's B'd per Gm. Casein
0.0	0.000	572.7	4.898	8.00×10^{-10}	0.0000	0.0000	$\times 10^{-3}$
0.1	0.001	653.1	6.257	1.827×10^{-9}	0.0000	0.0010	0
0.2	0.002	661.5	6.398	2.535×10^{-9}	0.0000	0.0020	10
0.3	0.003	671.6	6.569	3.76	0.0000	0.0030	20
0.4	0.004	678.7	6.689	4.953	0.0000	0.0040	30
0.5	0.005	686.7	6.825	6.759×10^{-9}	0.0000	0.0050	40
0.6	0.006	699.1	7.035	1.094×10^{-7}	0.0000	0.0060	50
0.7	0.007	727.3	7.511	3.279×10^{-7}	0.0000	0.0070	60
0.8	0.008	788.2	8.540	3.518×10^{-6}	0.0000	0.0080	70
0.9	0.009	833.5	9.306	2.050×10^{-6}	0.0000	0.0090	80
1.0	0.010	859.8	9.743	5.706	0.0000	0.0100	90
1.1	0.011	872.7	9.970	9.375×10^{-6}	0.0000	0.0110	100
1.2	0.012	881.5	10.115	1.311×10^{-4}	0.0000	0.0120	110
1.3	0.013	885.2	10.180	1.533	0.0000	0.0130	120
1.4	0.014	896.0	10.363	2.354	0.0001	0.0139	130
1.5	0.015	902.5	10.473	3.01	0.0002	0.0148	139
1.6	0.016	910.0	10.610	4.03	0.0004	0.0156	148
1.7	0.017	917.2	10.721	5.342	0.0005	0.0165	156
1.8	0.018	923.5	10.823	6.945	0.0008	0.0172	165
1.9	0.019	929.5	10.930	8.775×10^{-4}	0.0011	0.0179	172
2.0	0.020	940.0	11.035	1.10×10^{-3}	0.0014	0.0186	179
2.1	0.021	940.5	11.115	1.325	0.0018	0.0192	186
2.2	0.022	945.6	11.200	1.648	0.0021	0.0199	192
2.3	0.023	949.9	11.275	1.975	0.0028	0.0202	199
2.4	0.024	955.3	11.365	2.377	0.0035	0.0205	202
2.5	0.025	958.5	11.420	2.66	0.0040	0.0210	205
2.6	0.026	962.0	11.485	3.05	0.0046	0.0214	210
2.7	0.027	964.7	11.525	3.391	0.0052	0.0218	214
2.8	0.028	968.0	11.580	3.855	0.0059	0.0220	218
2.9	0.029	970.6	11.625	4.256	0.0065	0.0225	220
3.0	0.030	972.8	11.662	4.653×10^{-3}	0.0072	0.0228	225

clear, but it is certainly not explained on the basis of simple molecular division or peptization. Neither of these changes could alone increase the gram equivalents of alkali and acid bound per gram of protein. It is obvious that the instability of the highly unsaturated (with respect to base) paracaseinate, augmented by the higher temperatures employed in rennin clotting, is responsible for the greater sensitivity towards cations, and explains its coagulation.

Colloid Chemistry of Rennet Phenomenon

It is not possible to understand correctly the clotting of milk by rennin by considering it from any one point of view. Both phases of the phe-

TABLE VIII

ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT PARACASEIN SOLUTION PLUS VARYING AMOUNTS OF SODIUM HYDROXIDE

Cc. N/1 NaOH	N	E.M.F.	pH	cOH	N _i	n	Gm. Eqt's B'd per Gm. Para-casein
0.0	0.000	541.0	4.362	2.33×10^{-10}	0.0000	0.0000	$\times 10^{-5}$
0.1	0.001	584.4	5.096	1.312×10^{-9}	0.0000	0.0010	0
0.2	0.002	622.7	5.743	5.587×10^{-9}	0.0000	0.0020	10
0.3	0.003	638.2	6.005	1.028×10^{-8}	0.0000	0.0030	20
0.4	0.004	642.5	6.077	1.210×10^{-8}	0.0000	0.0040	30
0.5	0.005	651.1	6.223	1.687	0.0000	0.0050	40
0.6	0.006	659.2	6.360	2.318	0.0000	0.0060	50
0.7	0.007	666.4	6.481	3.068	0.0000	0.0070	60
0.8	0.008	674.0	6.610	4.130	0.0000	0.0080	70
0.9	0.009	685.7	6.808	6.495	0.0000	0.0090	80
1.0	0.010	691.0	6.898	7.970×10^{-8}	0.0000	0.0100	90
1.1	0.011	704.9	7.132	1.375×10^{-7}	0.0000	0.0110	100
1.2	0.012	728.5	7.531	3.310×10^{-7}	0.0000	0.0120	110
1.3	0.013	784.9	8.485	3.089×10^{-6}	0.0000	0.0130	120
1.4	0.014	811.6	8.936	8.728×10^{-6}	0.0000	0.0140	130
1.5	0.015	834.6	9.325	2.138×10^{-5}	0.0000	0.0150	140
1.6	0.016	850.3	9.590	3.938	0.0000	0.0160	150
1.7	0.017	864.8	9.830	6.924	0.0000	0.0170	160
1.8	0.018	875.6	10.019	1.024×10^{-4}	0.0000	0.0180	170
1.9	0.019	886.3	10.199	1.605×10^{-4}	0.0000	0.0190	180
2.0	0.020	893.3	10.318	2.104	0.0000	0.0200	190
2.1	0.021	897.5	10.388	2.477×10^{-4}	0.0001	0.0209	200
2.2	0.022	906.2	10.535	3.482	0.0003	0.0217	209
2.3	0.023	911.9	10.632	4.335	0.0004	0.0226	217
2.4	0.024	918.3	10.740	5.573	0.0005	0.0235	226
2.5	0.025	923.3	10.825	6.745	0.0008	0.0242	235
2.6	0.026	928.8	10.918	8.365×10^{-4}	0.0010	0.0250	242
2.7	0.027	934.2	11.008	1.035×10^{-3}	0.0013	0.0257	250
2.8	0.028	940.5	11.110	1.322×10^{-3}	0.0017	0.0263	257
2.9	0.029	944.8	11.185	1.558	0.0022	0.0268	263
3.0	0.030	950.5	11.284	1.952×10^{-3}	0.0028	0.0272	268
							272

nomenon, the action of the colloidal rennin on the caseinates, and the clotting of the resulting paracaseinate, are colloidal reactions.

At least three important questions stand out in connection with the rennin action, none of which can as yet be answered with complete satisfaction: What is the exact character of the colloidal calcium caseinate of milk? What is the nature of the change in this colloid which causes its increased affinity for cations? How does the colloidal rennin enzyme produce this change?

The ultramicroscopic evidence and the precipitation by electrolytes appear to indicate that calcium caseinates and paracaseinates are true suspensoids at the cH of milk. This is the fundamental error in Alex-

TABLE IX

ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT CASEIN SOLUTION PLUS VARYING AMOUNTS OF CALCIUM HYDROXIDE

Cc. N/1 Ca(OH) ₂	N	E.M.F.	pH	cOH	N ₁	n	Gm. Eq't's B'd per Gm. Casein
							× 10 ⁻³
0.0	0.000	563.7	4.915	5.624 × 10 ⁻¹⁰	0.0000	0.0000	0
0.1	0.001	599.8	5.356	2.300 × 10 ⁻⁹	0.0000	0.0010	10
0.2	0.002	620.7	5.710	5.177 × 10 ⁻⁹	0.0000	0.0020	20
0.3	0.003	636.4	5.975	9.514 × 10 ⁻⁹	0.0000	0.0030	30
0.4	0.004	645.2	6.123	1.350 × 10 ⁻⁸	0.0000	0.0040	40
0.5	0.005	659.7	6.355	2.363	0.0000	0.0050	50
0.6	0.006	668.9	6.557	3.386	0.0000	0.0060	60
0.7	0.007	694.7	6.960	9.237 × 10 ⁻⁸	0.0000	0.0070	70
0.8	0.008	723.7	7.450	2.857 × 10 ⁻⁷	0.0000	0.0080	80
0.9	0.009	762.4	8.105	1.290 × 10 ⁻⁶	0.0001	0.0089	89
1.0	0.010	801.9	8.765	5.996 × 10 ⁻⁶	0.0002	0.0098	98
1.1	0.011	824.2	9.149	1.430 × 10 ⁻⁵	0.0003	0.0107	107
1.2	0.012	837.0	9.366	2.350 × 10 ⁻⁵	0.0003	0.0117	117
1.3	0.013	845.2	9.504	3.234	0.0004	0.0126	126
1.4	0.014	857.2	9.707	5.154	0.0005	0.0135	135
1.5	0.015	862.0	9.787	6.210	0.0006	0.0144	144
1.6	0.016	871.3	9.945	8.920 × 10 ⁻⁵	0.0007	0.0153	153
1.7	0.017	879.5	10.085	1.250 × 10 ⁻⁴	0.0009	0.0161	161
1.8	0.018	887.0	10.211	1.650 × 10 ⁻⁴	0.0011	0.0169	169
1.9	0.019	893.7	10.320	2.192	0.0013	0.0177	177
2.0	0.020	900.7	10.443	2.798	0.0015	0.0185	185
2.1	0.021	905.0	10.515	3.320 × 10 ⁻⁴	0.0018	0.0192	192
2.2	0.022	911.2	10.620	4.214	0.0022	0.0198	198
2.3	0.023	916.0	10.700	5.095	0.0026	0.0204	204
2.4	0.024	920.7	10.780	6.102	0.0031	0.0209	209
2.5	0.025	924.7	10.849	7.144	0.0035	0.0215	215
2.6	0.026	928.8	10.918	8.365	0.0042	0.0218	218
2.7	0.027	932.6	10.985	9.688	0.0049	0.0221	221
2.8	0.028	936.1	11.040	1.114 × 10 ⁻³	0.0054	0.0226	226
2.9	0.029	939.0	11.090	1.240	0.0061	0.0229	229
3.0	0.030	942.5	11.149	1.425 × 10 ⁻³	0.0070	0.0230	230

ander's⁴⁶ theory. Suspensoids require special methods of preparation and are protected from coagulation by emulsoids; the colloids, calcium caseinate and calcium paracaseinate, are formed by simple dispersion in water and do not require emulsoid protection. On the other hand, these colloids cannot be classed with the hydrophils like gelatin, albumin, etc., which are nonsensitive to cations. Jablczynski⁴⁹ classifies slightly soluble dispersoids as colloids of the second order, and states⁵⁰ that their velocity of coagulation is not affected by a protective colloid like gum arabic. This appears to be a suitable classification for calcium caseinate and calcium paracaseinate although they are more soluble than

⁴⁶ Jablczynski, C. K., *Bull. Soc. Chim.* (4), 33, 1891 (1928).

⁵⁰ Jablczynski, C. K., *Bull. Soc. Chim.* (4), 35, 1286-1292 (1924).

TABLE X
ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT PARACASEIN SOLUTION
PLUS VARYING AMOUNTS OF CALCIUM HYDROXIDE

Cc. N/1 Ca(OH) ₂	N	E.M.F.	pH	cOH	N ₁	n	Gm. Eqt's B'd per Gm. Para- casein
							× 10 ³
0.0	0.000	544.0	4.412	2.610 × 10 ⁻¹⁰	0.0000	0.0000	0
0.1	0.001	563.0	4.668	5.470 × 10 ⁻¹⁰	0.0000	0.0000	10
0.2	0.002	579.4	5.011	1.036 × 10 ⁻⁹	0.0000	0.0000	20
0.3	0.003	593.3	5.246	1.781 × 10 ⁻⁹	0.0000	0.0000	30
0.4	0.004	606.4	5.468	2.968	0.0000	0.0000	40
0.5	0.005	613.6	5.589	3.920	0.0000	0.0000	50
0.6	0.006	623.1	5.750	5.673	0.0000	0.0000	60
0.7	0.007	632.0	5.900	8.030 × 10 ⁻⁹	0.0000	0.0000	70
0.8	0.008	640.0	6.035	1.100 × 10 ⁻⁸	0.0000	0.0000	80
0.9	0.009	647.8	6.168	1.488	0.0000	0.0000	90
1.0	0.010	655.9	6.304	2.033	0.0000	0.0000	100
1.1	0.011	663.6	6.435	2.752	0.0000	0.0000	110
1.2	0.012	674.1	6.612	4.146	0.0000	0.0000	120
1.3	0.013	684.0	6.779	6.100 × 10 ⁻⁸	0.0000	0.0000	130
1.4	0.014	698.5	7.025	1.070 × 10 ⁻⁷	0.0000	0.0000	140
1.5	0.015	764.0	8.132	1.370 × 10 ⁻⁶	0.0001	0.0149	149
1.6	0.016	820.6	9.088	1.240 × 10 ⁻⁶	0.0002	0.0158	158
1.7	0.017	832.1	9.283	1.938 × 10 ⁻⁶	0.0003	0.0167	167
1.8	0.018	842.0	9.450	2.860	0.0004	0.0176	176
1.9	0.019	849.4	9.575	3.806	0.0004	0.0186	186
2.0	0.020	860.3	9.760	5.822	0.0005	0.0195	195
2.1	0.021	866.1	9.857	7.037	0.0006	0.0204	204
2.2	0.022	873.7	9.986	9.809 × 10 ⁻⁶	0.0008	0.0212	212
2.3	0.023	979.3	10.081	1.215 × 10 ⁻⁴	0.0009	0.0221	221
2.4	0.024	887.8	10.225	1.702	0.0012	0.0228	228
2.5	0.025	893.7	10.325	2.136	0.0014	0.0236	236
2.6	0.026	901.7	10.460	2.914	0.0015	0.0245	245
2.7	0.027	907.9	10.565	3.7205	0.0016	0.0254	254
2.8	0.028	913.5	10.659	4.6125	0.0024	0.0256	256
2.9	0.029	921.2	10.789	6.215	0.0033	0.0257	257
3.0	0.030	927.3	10.892	7.8775 × 10 ⁻⁴	0.0041	0.0259	259

the substances which Jablczynski classifies as colloids of the second order (viz. AgCl); they undoubtedly owe their stability in pure water solely to their greater hydration. These colloids, therefore, appear to be striking examples of dispersoids with a nature intermediate between suspensoids and true emulsoids, dispersing in pure water like the latter, yet retaining their visibility in the ultramicroscope and their coagulability by cations.

There is much less certainty regarding the nature of the change in the particles of calcium caseinate which increases their affinity for cations with a corresponding rise in sensitivity. According to Michaelis⁵¹

⁵¹ Michaelis, L., Colloid Symposium Monograph II, 1925, pp. 1-15, Chemical Catalog Company.

TABLE XI

ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT CASEIN SOLUTION PLUS VARYING AMOUNTS OF HYDROCHLORIC ACID

Cc. 1.033 NHCl	N	E.M.F.	pH	cH	N ₁	n	Gm. Fqt's B'd per Gm. Casein
							× 10 ⁻³
0.00	0.0000	580.2	5.023	0.2240 × 10 ⁻³	0.0000	0.0000	0
0.05	0.0005	478.5	3.305	0.49575 × 10 ⁻³	0.0001	0.0004	4
0.10	0.0010	469.3	3.150	0.7119	0.0002	0.0008	8
0.15	0.0015	465.0	3.077	0.8380	0.0003	0.0012	12
0.20	0.0021	463.9	3.058	0.8754	0.0004	0.0017	17
0.25	0.0026	460.5	3.000	0.94925 × 10 ⁻³	0.0005	0.0021	21
0.30	0.0031	456.9	2.940	0.1145 × 10 ⁻³	0.0006	0.0025	25
0.35	0.0036	452.8	2.871	0.1351	0.0008	0.0028	28
0.40	0.0040	451.7	2.852	0.14115	0.0009	0.0031	31
0.45	0.0046	449.4	2.813	0.1538	0.0010	0.0036	36
0.50	0.0052	444.7	2.734	0.1851	0.0013	0.0039	39
0.55	0.0057	441.2	2.674	0.2114	0.0016	0.0041	41
0.60	0.0062	436.7	2.599	0.2520	0.0019	0.0043	43
0.65	0.0067	433.9	2.551	0.2811	0.0022	0.0045	45
0.70	0.0072	432.9	2.534	0.2922	0.0024	0.0048	48
0.75	0.0077	430.6	2.495	0.3200	0.0026	0.0051	51
0.80	0.0083	428.3	2.456	0.3498	0.0029	0.0054	54
0.85	0.0088	424.7	2.396	0.4028	0.0032	0.0056	56
0.90	0.0093	423.6	2.377	0.4204	0.0037	0.0056	56
0.95	0.0098	422.8	2.364	0.4335	0.0039	0.0059	59
1.00	0.0103	419.7	2.311	0.48905 × 10 ⁻³	0.0045	0.0058	58

interpretation of the current views on ionic effects on colloids the alteration in the particles should be confined to the electrical double layer. The difficulty in accepting this explanation lies in the fact that the alteration is permanent, pointing to definite molecular change. The nature of this change remains to be determined; but, as already pointed out, it cannot be a simple molecular division.

The question of the method by which minute amounts of colloidal enzyme can effect so profound a change in so short a time must at present remain on the unsatisfactory basis that it does so during a temporary adsorption on the surface of the calcium caseinate particles.

The second phase of the rennin phenomenon rests on much firmer colloidal grounds. The ultramicroscopic evidence and the accumulation of experimental observations definitely point to the conclusion that the rennet clot is an unstable, rapidly synerizing gel of amorphous particles of calcium paracaseinate, each retaining its identity. The laws governing the formation of this gel are identical with those recently summarized by Weiser⁵² for jellies formed by precipitation from a sol. The

⁵² Weiser, H. B., Colloid Symposium Monograph, 1st National Symposium 1923, pp. 38-61; Colloidal Behavior, edited by R. H. Bogue, I, pp. 377-409 (1924). McGraw-Hill Book Company.

TABLE XII

ELECTROMETRIC TITRATION OF 100 CC. OF A ONE PER CENT PARACASEIN SOLUTION PLUS VARYING AMOUNTS OF HYDROCHLORIC ACID

Cc. 1.033 NHCl	N	E.M.F.	pH	cH	N ₁	n	Gm. Eqt's B'd per Gm. Para- casein
							$\times 10^{-3}$
0.00	0.0000	562.7	4.729	0.1871×10^{-4}	0.0000	0.0000	0
0.05	0.0005	491.5	3.524	0.2994×10^{-3}	0.0000	0.0005	5
0.10	0.0010	486.2	3.435	0.2676	0.0000	0.0010	10
0.15	0.0015	481.8	3.360	0.4364	0.0000	0.0015	15
0.20	0.0021	477.3	3.285	0.51915	0.000	0.0020	20
0.25	0.0026	473.8	3.225	0.5956	0.0001	0.0025	25
0.30	0.0031	470.5	3.165	0.6765	0.00015	0.0029	29
0.35	0.0036	467.5	3.120	0.7605	0.0002	0.0034	34
0.40	0.0040	465.2	3.080	0.8317	0.0003	0.0037	37
0.45	0.0046	462.5	3.035	0.92425×10^{-3}	0.0004	0.0042	42
0.50	0.0052	459.4	2.982	0.10438×10^{-2}	0.0006	0.0046	46
0.55	0.0057	457.5	2.950	0.1100	0.0006	0.0051	51
0.60	0.0062	454.2	2.894	0.1265	0.0008	0.0054	54
0.65	0.0067	450.2	2.825	0.1484	0.0009	0.0058	58
0.70	0.0072	447.1	2.774	0.16835	0.0011	0.0061	61
0.75	0.0078	443.9	2.720	0.1907	0.0013	0.0065	65
0.80	0.0083	442.4	2.695	0.2018	0.0014	0.0069	69
0.85	0.0093	435.2	2.573	0.2670	0.0021	0.0072	72
0.95	0.0098	431.1	2.490	0.3138	0.0025	0.0073	73

type of precipitation reaction is the one involving the effect of valency of the precipitating ion, which was clearly shown first by Loevenhart, and which is apparently explained best by the current views regarding the electrostatic effects of ions on colloids. There is also a definite temperature range for the calcium paracaseinate gel. Temperature also is a factor in the rapidity of its syneresis. We have not yet determined the calcium combining capacity of casein and paracasein at the optimum clotting temperature, but it is reasonable to suppose that the same relationship holds as at room temperature. Bang¹³ believed that the affinity for calcium increases with rise of temperature. This would account for the greater instability in the presence of soluble calcium salts. The rule seems to be that the greater the calcium binding capacity of the particles is and the farther removed they are from saturation with calcium, the less calcium ions are required to neutralize their electrical charges. This undoubtedly explains in part why sodium ions will clot the calcium paracaseinate formed in milk by rennin action.

As already pointed out, Hammarsten¹⁴ held that calcium paracaseinate solutions never give a gel with NaCl but only a flocculent precipitate, while dialyzed milk after treatment with rennin gives a gel with NaCl. Hammarsten used this as an argument for his theory that casein

exists in milk as a calcium caseinate-calcium phosphate complex. The more commonly held view is that calcium caseinate holds a colloidal calcium phosphate in suspension. Weiser⁵² states that the velocity of precipitation determines whether a gel or a gelatinous precipitate will form. It may be expected that the presence of a suspensoid which is not altered by rennin will retard sufficiently the rate of precipitation of the calcium paracaseinate to account for the gel. We have found that calcium paracaseinate solutions made alkaline with $\text{Ca}(\text{OH})_2$ and neutralized to pH 6.5 with H_3PO_4 give gels and not gelatinous precipitates with NaCl at 40° C. Much more conclusive proof that the colloidal calcium phosphate in milk is a factor in gel formation with rennin was secured by comparing the effects of NaCl at clotting temperatures on mixtures of calcium paracaseinate and gelatin on the one hand, and calcium paracaseinate and nearly neutral gelatin suspensions of colloidal calcium phosphate on the other hand. Each mixture contained the same percentage of the same kind of gelatin. On adding 0.5 per cent NaCl the paracasein-gelatin mixture gave an extremely weak clot which settled very rapidly as a flaky precipitate; the paracasein-colloidal phosphate mixture, however, gave fairly good clots which showed definite syneresis and maintained a bulky curd even after standing for an hour at clotting temperature.

In addition to explaining Hammarsten's results on the basis of the more prevailing ideas regarding the colloidal status of the calcium phosphate of milk, our findings suggest a new method of gel formation; namely, the precipitation of micellæ by cations in the presence of a suspensoid which is peptized by the precipitating ion.

Another important phase of the colloid chemistry of rennet coagulation is the detrimental effect of heat as shown by the fact that boiled milk no longer gives a normal gel but instead a heavy gelatinous precipitate. The present theories explaining this result are not satisfactory. They are based on the circumstantial evidence that, inasmuch as CaCl_2 restores to a large extent the original properties of the milk, heat must therefore remove soluble calcium salts necessary for a normal clot. We are at present engaged in investigating this problem and have reason to believe that the correct explanation will be disclosed by our study.

University of Minnesota,
Minneapolis, Minn.

THE COLLOID CHEMISTRY OF PROTOPLASM

BY L. V. HEILBRUNN

The investigation of the colloidal properties of any material is in itself no simple matter. With protoplasm or living substance the problem becomes infinitely more difficult and more complex. The reasons are obvious. In the first place protoplasm is extremely sensitive. Touch it and it becomes materially changed, treat it at all harshly and it dies. Nor is this all. The chief difficulty in working with protoplasm is that it is so inaccessible. It is contained in, or forms, cells of tiny dimensions, on the average not over a hundredth of a millimeter in diameter, and it must be studied in this cellular state if it is to be studied at all. And yet tiny as these cells are, they contain a wide variety of complex chemical substances, combined and arranged we know not how.

Small wonder then that the biologist has in the past been content to speculate as to the colloidal changes in protoplasm. Nor have his speculations always been wise ones. It has been and still is the custom in biological thought to seek an analogy between protoplasm and various types of lyophilic colloids. Of these gelatin has been the favorite, and many a physiologist has performed a series of experiments on gelatin with the apparent assumption that his results might be applied directly to protoplasm. Nothing could be much farther from the truth, as will, I think, be demonstrated in the course of this discussion. On the very face of it the analogy is a poor one. Simple microscopical examination reveals the well-known fact that protoplasm in general is a suspension and possibly also an emulsion. Its behavior would then, to some extent at least, be governed by the laws which govern the behavior of suspensions and emulsions, and these are, as has frequently been pointed out, much more nearly those which apply to lyophobic colloids than to lyophilic colloids.

If we are to obtain any real information about protoplasm we must work directly with it. Analogy is often worse than useless. Some day perhaps, when we understand more thoroughly the physico-chemical make-up of living material, we may be able to prepare substances or mixtures of substances which behave like it. But to choose analogies blindly is a dangerous practice.

As with all scientific inquiry the investigation of the colloidal properties of protoplasm depends on the development of some reliable method

of measurement. Mere microscopic examination in the past has proved futile. By looking at cells it is not possible to decide even the simplest details as to the physical properties of their protoplasm. Nor does the introduction of a needle into the cell very materially help the observa-

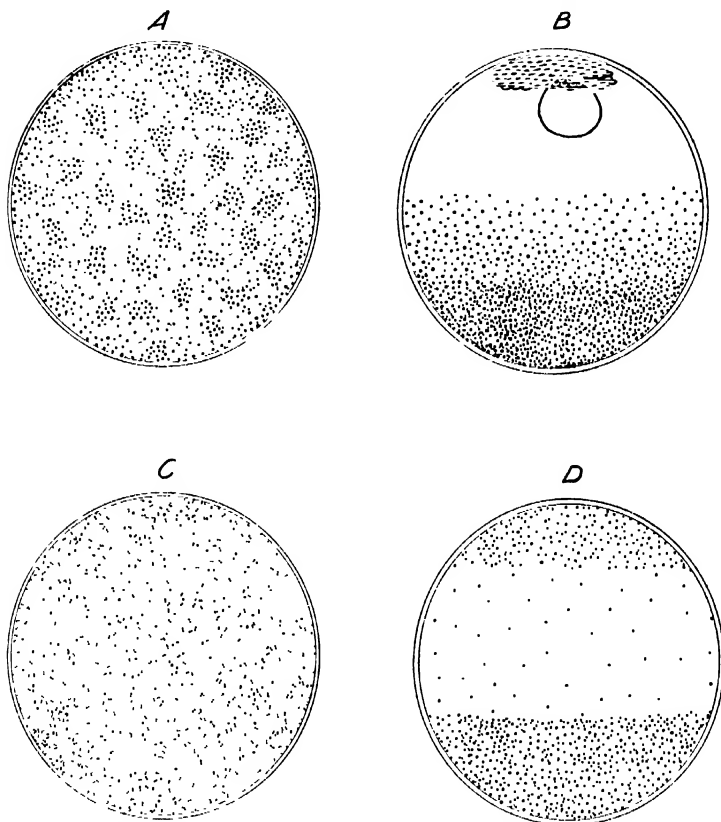


FIG. 1.—Sea-urchin and Cumingia Egg Cells before and after centrifugal treatment.

tion, although it does injure the protoplasm. Fortunately within the last ten or eleven years at least two methods have been devised which make possible a determination of the viscosity of the protoplasm without destroying or even injuring the cell. For a while, therefore, viscosity measurement may be the only key to the solution of the puzzling problems involved in the colloid chemistry of protoplasm, much as

Graham supposed that it would be the main key to the problems of colloid chemistry in general.

Both methods of protoplasmic viscosity measurement are based on the same principle. Both apply Stokes' law for the movement of a spherical particle through a fluid. The speed of movement of granules or other particles in the protoplasm is taken as an index of the viscosity. In plant cells the force of gravity is often sufficient to cause a movement of starch grains. The granules of animal cells ordinarily do not move under the influence of gravity, in these cells centrifugal force can be used to push the granules through the protoplasm. Recently the movement of metallic particles under the influence of a magnetic force has also been used in viscosity measurement,¹ but this method, as pointed out by its discoverer, Heilbronn, is only of limited applicability, for the introduction of metallic particles into a cell is not usually possible without serious injury.

For relative measurements of protoplasmic viscosity, Stokes' law can generally be used without correction. For the measurement of absolute viscosity, various corrections should in some instances be applied. These include corrections for the presence of a cell membrane, for the presence of many granules rather than a single granule, for collisions between large and small granules when differences in size occur.

The technique of viscosity measurement has been considered in various published papers. These have been recently summarized by Weber.² In working with animal cells, an ordinary hand centrifuge may be used. Fig. 1 shows the appearance of two cells before and after centrifugal treatment. In this figure A and B refer to the sea-urchin egg, C and D to the egg of the clam *Cumingia*. In both instances following centrifugal treatment there is an accumulation of granules lighter than the protoplasm at one pole of the egg, whereas the heavier granules move to the opposite pole. The sea-urchin egg differs from the *Cumingia* egg in having heavier granules of two different sizes, these are distributed in two distinct zones after centrifuging.

When cells are centrifuged the speed of movement of the granules varies with the fluidity of the protoplasm or inversely with the viscosity. This is in accordance with Stokes' law which may be stated in the following form:

$$V = \frac{2cg(\sigma - \rho)a^2}{9\eta}$$

in which V is the velocity of the granules, c the centrifugal force in terms of gravity, σ the specific gravity of the granules, ρ the specific gravity of the material between the granules, a their radius, and η the

viscosity of the medium through which the granules travel. For relative measurements it can usually be assumed that σ and ρ remain constant, then if a remains constant the velocity V gives a measure of the fluidity.

One disadvantage in the use of viscosity measurement in the study of suspensions and other colloids is the unfortunate fact that various types of measurement do not always agree. Moreover there is often a disagreement in the values obtained at different rates of shear (cf. Humphrey and Hatschek).⁸ In the one type of protoplasm that has been investigated, the rate of shear, or rather the rate of movement of the granules through the protoplasm, does not have any great influence. This is shown by the following table in which *Cumingia* eggs were centrifuged at different speeds. The first column gives the centrifugal force in terms of gravity, the second column shows the viscosity in arbitrary units. The measurements are not accurate, especially for high centrifugal speeds, and the divergent value for the highest centrifugal speed is certainly within the range of error of the method.

Centrifugal Force	Viscosity
310.5.....	3.5
552.....	3.7
1242.....	3.5
4968.....	3

A remarkable advantage of the centrifugal method lies in the fact that its use is not restricted to isolated cells. Even those cells in the interior of an animal can be investigated. It is possible to centrifuge entire organs, and indeed many animals can be centrifuged whole. Their tissues can then be prepared with the ordinary methods of histological study, sectioned and examined at leisure. This method has been tried to some extent, but so far the results have been meager. It may be necessary to use higher centrifugal speeds than those heretofore employed.

Our discussion will therefore be limited to the case of isolated cells. With these, measurement is far more rapid, as the results of centrifugal tests can be observed directly. But even so it is a slow task, each determination requiring a number of tests before one can decide on the exact number of seconds of centrifugal treatment required to move the granules a given distance through the cell.

And now for the facts. That the story I have to tell is a painfully brief one, I realize only too well. But I ask your indulgence, for as I pointed out at the start, the investigation of protoplasm from a physical standpoint is beset with most unusual difficulties.

The first point to be settled is the important question as to whether protoplasm is a sol or a gel. This is a question that is commonly answered in various textbooks. The difficulty is that sometimes it is an-

swered in one way—sometimes in another. The concept of a gel may vary to some extent with different colloid chemists, but in general the term implies a material with form, or elasticity, a material so viscous that it flows, if at all, with extreme slowness. Its viscosity with ordinary measurement is practically infinity. Certainly in those cases in which reliable measurements of the absolute viscosity have been made, protoplasm is far from being a stiff gel. For various plants Heilbronn⁴ has determined the viscosity to be about 10 to 20 times that of water. Compare this with the huge values given for the viscosity of gelling sols,⁵ and it is obvious that the protoplasm of plants in as far as it has been investigated is in no sense a rigid gel.

Up to the present no adequate measurements have been published for animal cells. If the centrifuge method is to be used for a determination of the absolute viscosity of protoplasm, it is essential to know the specific gravity of the granules of the protoplasm as well as the specific gravity of the medium in which the granules lie. As a matter of fact it has been found that both these quantities can be determined with sufficient accuracy. Details of the methods involved are being published elsewhere. Suffice to say that when the proper values are substituted into the formula given above, the viscosity of *Cumingia* egg protoplasm is found to be about 4 times that of water. In the *Cumingia* egg the determination of the absolute viscosity is much more simple than in the case of the sea-urchin egg. The larger number of granules in this egg makes necessary a correction for Stokes' formula, inasmuch as this formula was derived for a single sphere moving through a fluid. Fortunately such a correction has been worked out by Cunningham.⁶ Moreover, in the sea-urchin egg the heavier granules are of two sizes, they tend to collide and the larger ones are retarded. But in spite of all these complexities it can be shown that the protoplasmic viscosity of the sea-urchin egg is of the same order of magnitude as that of the *Cumingia* egg. In both instances the material surrounding the granules is a fluid sol. It is hard to realize that the protoplasm of living cells may be two hundred times as fluid as glycerine, four times as fluid as sulfuric acid, but the value obtained for the intergranular material of the *Cumingia* egg is about what one would expect for a protein sol.

It has probably occurred to many of you that there are in reality two viscosities to be considered. We can think of protoplasm as the material surrounding the granules, or, and this seems perhaps wiser, we can think of protoplasm as the entire mass—granules and all. At any rate, in whichever way we frame our definition, we must seek to set a value both for the fluid between the granules and for the entire mass. So far only the first of these quantities has been discussed. The viscosity of the total mass of the protoplasm will obviously increase with

the amount of suspended material. It will doubtless be much greater in those cells more closely packed with granules.

To estimate the viscosity of the entire protoplasm we can resort to the formulas of Einstein⁷ and Hatschek⁸ for the viscosity of suspensions,

$$\eta_c = \eta_0(1 + kf)$$

in which η_c is the viscosity of the suspension, η_0 the viscosity of the dispersion medium of the suspension, f the ratio of the volume of suspended particles to the total volume of the suspension, and k is a constant which Einstein gives as 2.5 and Hatschek as 4.5. For the sea-urchin egg f is about 0.25, and for the Cumingia egg it is decidedly less than this. The viscosity of the entire mass of the protoplasm is therefore not much more than twice that of the intergranular fluid, if the formulas of Einstein and Hatschek are correct.

As a matter of fact these formulas hold only approximately at best. Bingham⁹ has suggested another formula which apparently gives better results. To use this formula it is necessary to know how great a concentration of suspended material is necessary to reduce the fluidity to zero. At first sight it seems impossible to apply this formula, for it is not practicable to introduce more and more granules until the point of zero fluidity is reached. On the other hand it is a comparatively simple matter to reduce the volume of the cell osmotically until the granules no longer move at all when the cells are subjected to centrifugal force. As yet no systematic studies of this sort have been made, but from previous determinations of the viscosity of cells in media of increased salt content, it seems certain that the values obtained by the use of Bingham's formula are of the same order of magnitude as those obtained with the Einstein-Hatschek formula. Similar values are also obtained with the formula used by Hess for suspensions of blood cells.¹⁰

It should be pointed out that none of the above formulæ takes account of the electrical charges of the granules. The effect of such a charge is given in a formula derived by von Smoluchowski.¹¹ It is important only when the granules are very small, and the specific conductivity of the medium is also small. Neither condition would apply in the case we are considering.

The protoplasm of the Cumingia egg has a viscosity about like that found for various plant cells by Heilbronn.⁴ True his values are slightly higher, but in his use of Stokes' law he does not take account of one or two corrections which might tend to lower the viscosity value.

It must not however be supposed that all protoplasm is as highly fluid as that of Cumingia or of plant cells. A student of mine, Miss Dorothy Fetter, has determined the protoplasmic viscosity of the proto-

zöon paramecium, by measuring the speed of movement of starch granules and iron particles taken into the paramecium cell as food. She arrives at a value of about 8000 for the viscosity of the entire protoplasm, granules and all. Perhaps other cells have protoplasm even more viscous. Only measurement can decide.

It must be remembered too that the protoplasmic viscosity varies with the functional activity of the cell. Thus in the *Cumingia* egg during cell division the viscosity may rise to 6 or 8 times the value previously given.¹²

But in so far as our knowledge goes, protoplasm is typically fluid and there is little or no justification for the frequent assumption that it is a gel.

Not all of the cell is fluid. This would be impossible if the cell were to remain isolated from the surrounding medium. It is surrounded by a thin membrane whose stiffness indicates it to be a rigid gel. This is the osmotic membrane, the so-called plasma membrane of the cell (cf. Heilbrunn '15¹³). It is an extremely important part of the cell, even though it constitutes only a relatively small part of its total volume.

If the membrane of the cell is ruptured and the interior protoplasm is allowed to come in contact with the surrounding medium, the naked protoplasm immediately surrounds itself with a new osmotic membrane which has all the properties of the old one, and is evidently the result of some precipitation process.

We conceive of protoplasm therefore as consisting of an interior mass of fluid surrounded by a precipitation membrane of the Traube type. The interior fluid is a protein sol and contains various granules and droplets suspended in it.

It is apparent that the granules and droplets of the protoplasm, and perhaps also the micellæ of the protein sol, are the seat of an electric charge. This charge most certainly is of importance in any consideration of the colloidal properties of protoplasm, and it doubtless determines to a considerable extent both the physical and the biological behavior of the living material. Is the charge positive or negative? To the colloid chemist this seems an absurdly simple problem, but it is not. It cannot be solved by the simple test of determining how the cell behaves when it is placed between two electrodes. The direction in which cells migrate under the influence of an electric current is largely the result of the charge at the surface. But we are interested not only in the surface charge but also in the charge on the particles in the interior. There is no reason why the charge at surface and interior should be of the same sign.

It is advisable therefore to use a roundabout method. If the particles or granules of protoplasm are allowed to come in contact with various cations of differing valences, it is apparent from well known

principles of colloid chemistry that bivalent cations would be much more effective than monovalent cations in neutralizing an electric charge if the charge were originally negative. They would then exert a greater precipitating or coagulative action. On the other hand if the normal charge were originally positive, an excess of bivalent ions, or an increase in the percentage of bivalent ions over that normally present, would tend to prevent coagulation. Here then is a method of test, for cells can be treated with various cations and the effect on the viscosity can then be noted. It was found for egg cells and for the cells of the protozoön *Stentor*, that when they were removed from their normal medium which contains salts with both monovalent and bivalent cations, and were placed in isotonic solutions of magnesium or calcium salts at constant pH, the protoplasm instead of coagulating became more fluid.¹⁴ Apparently the interior protoplasm contains dispersed material with a positive charge. But not the thin membrane at the surface. The magnesium and calcium salts tend for a time at least to make this membrane more rigid. Presumably the particles in the surface membrane have a negative charge.

The action of calcium ions is more pronounced than that of magnesium ions, apparently because of a greater adsorption of the calcium. Even more striking results are obtained with trivalent ions such as cerium and aluminum. It is difficult to work with these ions because of the acidity of solutions of their salts. However it was possible to show under conditions in which the H ion concentration was controlled, that cerium and aluminum ions act like the bivalent cations only much more powerfully. Even in concentrations as low as $m/25000$, aluminum chloride had a very marked effect on the fluidity of the interior protoplasm, sometimes reducing it to one-fourth of its original value. We are led therefore to conclude that the interior of the protoplasm contains particles with a positive charge.

And there is other evidence in support of this view. When various types of cells are placed in an electric current, it has been found that small cells generally migrate to the anode as though they were negatively charged, but that with increase in size of the cell there is a much greater tendency either for migration of the entire cell toward the cathode or for a bulging out of the protoplasm in that direction. These are old facts but were never properly interpreted by the men who observed them. It seems a reasonable interpretation to assume that in the case of the small cells their migration in the electric current is determined entirely by their surface charges; with increase in the size of the cell the interior mass becomes not only actually but also relatively larger and the positive charges in the interior tend to send cells or protoplasm to the cathode. For references to the literature on the migration of cells in an electric current, as well as for further argument to show that the

interior of the cell is filled with positively charged particles, whereas the surface membrane is charged negatively, see Heilbrunn '23.¹⁴

If this concept is the correct one, it will have a profound influence on various types of physiological theory. At the present time there are a number of widely accepted biological hypotheses which are built around a postulated electrical organization of the cell for which there is no direct evidence, little evidence indeed outside of the theories themselves.

Moreover the facts with regard to the action of various cations on protoplasm are in themselves important. For a long time one of the most engaging mysteries of biology has been the well-nigh universal fact that sodium and potassium ions on the one hand, magnesium and calcium ions on the other, have opposite effects on protoplasm, and that both types of ions are necessary for the life and proper functioning of the cell. In recent years it has been apparent to some workers at least that the explanation of this striking antagonism was in some way a colloid chemical one. Now and again biologists and chemists have studied the effects of sodium and calcium ions on colloidal media they thought comparable to protoplasm. The demonstration of an actual effect of these ions on the protoplasm itself is most certainly a step in advance. Especially in view of the fact that none of the workers who argued as to the effect of ions on protoplasm from observations on supposedly similar material ever reached what is apparently the true explanation.

Before passing on to other topics it may be well to add a word or two as to the possible origin of the negative charge which so universally occurs at the surface of living protoplasm. There is evidence that the charge is at least in part due to the diffusion of carbonic acid from the interior of the cell. In practically all living material the activity of the cell is bound up with a steady production of carbonic acid which is passed out to the exterior. Apparently the quantity of carbon dioxide passing through the surface membrane of the cell determines the magnitude of the electric charge that this membrane possesses. If we assume that the H ion is less able to penetrate the membrane than the carbonate ion, and there is experimental evidence in favor of this assumption (see, for example, Smith¹⁵), then it is evident that the diffusion of carbonic acid from the cell would tend to give a negative charge to the outer surface (cf. Ostwald¹⁶). The magnitude of the charge would depend on the amount of carbonic acid being given off by the cell. If this reasoning is correct we would expect that those parts of an animal or a tissue which are most active, and which both on theoretical and experimental grounds we know to give off the most carbon dioxide, would have a lower potential at their surface than less active parts or regions. This we know to be true. Many cases could

be cited. One example is that of the hydroids. In these animals Miss Hyman has shown that the more terminal portion has a higher rate of carbon dioxide production and a lower surface potential.¹⁷ And a similar relation holds true for the active and inactive parts of nerve and muscle. For additional cases of the same sort and for references to the literature the interesting paper of Hyman and Bellamy¹⁸ may be consulted.

It might be possible to go farther and to show how other biological phenomena are related and to some extent explained by our concept

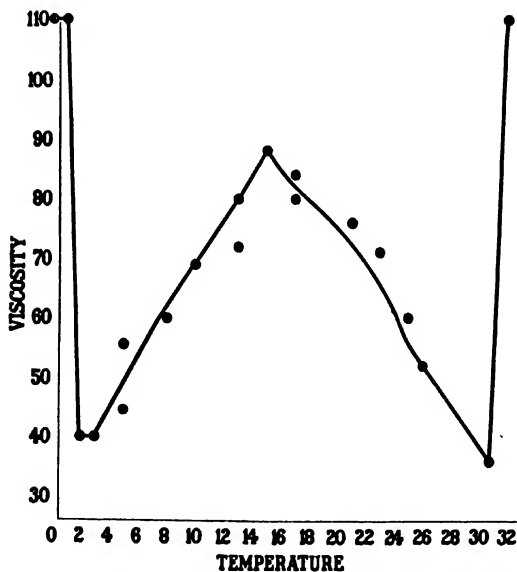


FIG. 2.—The Viscosity of *Cumingia* Egg Protoplasm at various temperatures.

of the electrical charges of a cell. But it is my purpose to hurry over these biological topics and to confine my talk for the most part to the colloidal properties of protoplasm, treating the subject as though the living substance were a material interesting only from a physico-chemical standpoint.

Various colloids differ in their relation to temperature. It is doubtful if in any known colloid the behavior is as complex as is that of protoplasm. A glance at the temperature viscosity curve¹⁹ will help to bear out this statement. Fig. 2 shows the viscosity of *Cumingia* egg protoplasm at various temperatures. The viscosity values are in arbitrary units, and they are not highly accurate. It will be seen that the viscosity

of the protoplasm goes through a maximum at 15° . This is a most unusual condition and it is not confined to the protoplasm of marine eggs. Heilbronn¹ has shown the same sort of a maximum in the protoplasm of slime molds. Since the publication of a temperature viscosity curve for protoplasm a year ago, at least two papers have been published which indicate that various physiological phenomena may be asso-

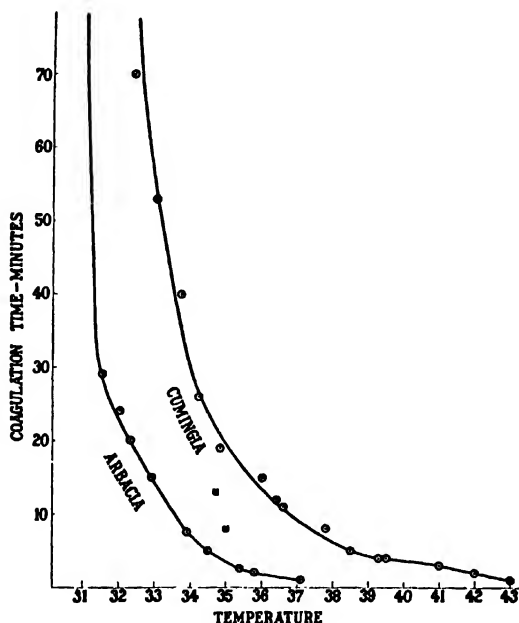


FIG. 3.—The Time of Heat Coagulation of Cumingia Egg Protoplasm at various temperatures.

ciated with the peculiar maximum in viscosity at 15° .²⁰ Moreover it has been known for a long time that the temperature coefficient of various biological processes may go through a sharp change at around 15° .²¹ Perhaps the viscosity change at this temperature contributes in some way to the explanation of this interesting fact. The sudden increase in viscosity at 1° is really greater than that shown in the graph, the determination at this point was subject to an error which tended to decrease the true value. Perhaps this unusual coagulative change at temperatures just above the freezing point may be responsible for the remark-

able fact that some plants give the appearance of being frost-killed at temperatures above freezing.²² At about 32° coagulation sets in.

Heat coagulation of protoplasm is not the same phenomenon as heat coagulation of proteins, in spite of the fact that the temperature coefficient of the two processes is very similar. Fig. 3 shows the coagulation time of the protoplasm of the *Cumingia* egg at various temperatures.²⁴ The curve shown in the figure is very similar to the curve that may be obtained for a protein (cf. Buglia²⁴ or the tables of Chick and Martin²⁵). But it will be noticed that the coagulation of protoplasm occurs at much lower temperatures than is typical for the heat coagulation of proteins. Pure proteins commonly coagulate at temperatures above 50° and the description of heat coagulation of proteins at temperatures of 30° to 40° is very doubtful. Moreover the heat coagulation of protoplasm differs, in one important respect from the heat coagulation of proteins. It is reversible, at least for a time. This is an essential difference, for when proteins are coagulated by heat the process is completely irreversible.

There is some evidence that the heat coagulation of protoplasm is due primarily to an alteration of the fatty constituents of the cell. Under the microscope these can be seen to be affected as the temperature is raised to about the point of heat coagulation. They tend to dissolve. Moreover by the addition of a small percentage of ether the heat coagulation of protoplasm can be very materially hastened. Finally it may be of significance that animals which are able to resist higher temperatures are in general provided with fats of higher melting points than those which succumb to more moderate temperatures. Thus fishes are killed at comparatively low temperatures and it is interesting to note that the fatty materials in the bodies of fishes, and perhaps also in their protoplasm, have a relatively low melting point. All these facts taken together are an indication that the lipoid or fatty constituent of protoplasm may play an important part in the coagulation of the living substance by heat. But what this part is only future investigation can decide.

There is other evidence besides that of the effect of temperature to indicate that the fatty constituents of the cell are of great importance in determining both the physical and the biological behavior of the protoplasm. Most fat solvents have a remarkable effect on living things. They stop various manifestations of life without causing death. In other words they have the power of reversibly inhibiting living processes. The fact that ether, chloroform, and other fat solvents can inhibit that activity of the brain cells which we call consciousness is of everlasting importance to the practice of medicine and more especially surgery. But the anesthetic action of ether is not confined to the effect on consciousness, nor is it restricted to man. All animals, from the lowest

to the highest, are anesthetized by ether, and the percentage of ether that is effective is remarkably constant for widely different sorts of organisms.

The problem of anesthesia is then a problem as wide as biology itself. Claude Bernard, who was perhaps the founder of the science of general physiology, thought of anesthesia as its most important problem. He himself was inclined to search for a colloid chemical explanation, for he believed that various anesthetics produced a reversible "semi-coagulation" in the protoplasm. Since Claude Bernard's time various workers have claimed that the fat solvent anesthetics cause a coagulation in the protoplasm. Most of these workers have studied the effects of ether and similar substances on protein solutions. Other workers have found the opposite sort of an effect with other inanimate preparations. The first real work on protoplasm was done by the German botanist Heilbronn. He studied the effect of ether on the protoplasmic viscosity of the cells of the horse-bean *Vicia faba*.²⁶ He found that dilute ether solutions caused a decrease in viscosity, more concentrated solutions a pronounced increase. Both of these changes were reversible. Following the work of Heilbronn, I studied the effect of fat solvents on the protoplasm of sea-urchin eggs.²⁷ My results are in the main similar. Solutions of a dozen or more fat solvents were tried. All of them in certain dilute concentrations caused a marked liquefaction of the protoplasm. This was reversible and resulted in no serious injury to the cells. Slightly higher concentrations produced a coagulation, but this was not reversible and it resulted in the death of the cell. There is thus a slight difference in the behavior of plant and animal cells. In both instances more dilute ether solutions cause a liquefaction or more properly a decrease in viscosity which is reversible, but the coagulation caused by more concentrated solutions is reversible for plant cells while it is irreversible for certain animal cells. Since the earlier work of Heilbronn, Weber²⁸ has shown that the protoplasm of the alga *Spirogyra* behaves toward ether like that of the higher plant.

Within the last year²⁹ I have gone over my older work on the effect of ether on sea-urchin eggs, and have obtained somewhat more quantitative results. These are not so easy to obtain as might be supposed. Each viscosity test is in reality an entire series of tests, as explained previously. The following table shows the effect of 2½ and 3% ether on unfertilized sea-urchin eggs. The third column gives the relative viscosity of the etherized eggs as compared to that of the control eggs shown in the fourth column.

From the table it can be seen that eggs in 2½ to 3% ether have their viscosity reduced to about one-half of its original value. In these solutions the viscosity of the protoplasm does not remain con-

Per Cent Ether	Exposure, Minutes	Viscosity Etherized Eggs	Viscosity Control Eggs	Temperature
2.5	11	10	25	23
2.5	4	15	25	—
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8.5	20	40	23
3	3	15	30	25.3

stant, it tends to become lower and lower until suddenly a turning point is reached and coagulation occurs. Thus in 3% ether at 22°, coagulation occurred about 26 minutes after the cells were introduced into the ether solution. Higher concentrations produce coagulation more rapidly. Once it has occurred, the egg is never able to return to its original fluid state.

The above results were obtained with unfertilized eggs. An even more pronounced effect may be noted if the eggs are fertilized before being treated with ether. In sea-urchin eggs the entrance of the spermatozoon is soon followed by a very pronounced increase in the viscosity of the protoplasm.³⁰ But if the eggs after fertilization are placed in dilute ether solutions there is no such increase in viscosity. Moreover if fertilized eggs which have already undergone an increase in viscosity are placed in dilute ether solutions, the viscosity of the protoplasm of these eggs becomes as low or even lower than if the eggs had never been fertilized. In these eggs the viscosity is reduced to a sixth or even an eighth of its original value.

Why or how ether produces its effect on protoplasmic viscosity is still a mystery. Thomas³¹ found that anesthetics caused changes in the viscosity of lecithin suspensions and long before this Höber and Gordon³² showed that ether vapor tended to prevent the precipitation of a lecithin suspension by salts. Whether these observations can be correlated with the effect of anesthetics on protoplasm is somewhat doubtful. An interesting point which may have bearing on the subject is the fact that distilled water tends to produce the same effect as fat solvents. If the sea-water in which the sea-urchin eggs are contained is diluted with distilled water there is a sharp decrease in the viscosity of the protoplasm. But when more and more distilled water is added there is a turning point and coagulation occurs.²⁷

Whatever the physical explanation of the ether effect may be, it seems certain that the observed facts have an important bearing on the biological theory of anesthesia. If it is true that ether and similar anesthetics can without injury prevent colloidal changes in protoplasm, it seems likely that this property is the real basis of their anesthetic

action. Anesthesia caused by fat solvents is apparently due to a prevention or an inhibition of coagulative change. If we make this assumption we must also conclude that the activity of a cell is bound up with coagulative changes in the protoplasm.

This as a matter of fact is our firm belief. It is not a new idea. As soon as it became at all apparent that the living material was a fluid or a semi-fluid, as soon indeed as biologists began to think of it in physical terms, they suggested that in its various activities it might suffer coagulations like those which had previously been observed in such animal fluids as blood and milk. But it is easier to demonstrate the coagulation of blood or milk than the coagulation of protoplasm. Only with the development of reliable methods for viscosity determination within the living cell has it been possible to show conclusively that at least one process is associated with a coagulative change or at any rate a change involving a great increase in viscosity. Thus it was found in 1915¹² that the early stages of cell division in sea-urchin eggs were accompanied by a sharp increase in viscosity. This knowledge has now been confirmed for plant cells.¹³

Doubtless in the future many processes other than cell division will be found to be associated with colloidal changes in the protoplasm. Presumably muscular contraction involves some colloidal changes. At least such a change is postulated in many of the theories that have been proposed to explain the physical behavior of muscle. But whereas some physiologists hold, or have held, that muscular contraction is the result of a coagulation of the protoplasm, others argue that it is due to a colloidal swelling or liquefaction. Unfortunately muscle cells are not very suitable for a direct determination of the protoplasmic viscosity.

Perhaps it may be worth our while to reason about the colloidal phenomena of muscle and other living structures in an indirect way. If the effects of chemicals on protoplasm are the same for various sorts of protoplasm, and we have reason to believe within fairly wide limits that they are, then we can apply our knowledge of such effects to the interpretation of biological processes, even in those instances where there are at present technical difficulties in the way of a direct determination of the protoplasmic viscosity. Thus for example if it is true, and I think it is, that a higher proportion of sodium ions in the medium surrounding a cell or group of cells tends to produce an increased viscosity and that an increase in the proportion of calcium ions tends to have the opposite effect, then if it is found that sodium ions stimulate a muscle to contraction and that calcium ions tend to prevent such a contraction, we have reason for assuming that the contraction process is in some way associated with increased viscosity of the protoplasm. Actually it has been known for a long time that sodium ions do stimulate

muscle cells to contract and that the addition of calcium ions tends to prevent this action.³⁴

This is but one example of the manner in which the knowledge of the colloid chemistry of protoplasm may be used. Perhaps by similar methods of reasoning other living processes can to some extent be analyzed. But only the future can tell to what extent the method is useful, if at all.

Before concluding the discussion of the colloidal properties of protoplasm, a word or two might be added as to the effect of acids and alkalies on protoplasmic viscosity. This study has not been pushed very far as there are various difficulties which retard rapid progress. But it may be said in general that both acids and alkalies produce irreversible coagulation. Among acids the behavior of carbonic acid is unique and especially interesting. Jacobs³⁵ has shown that this acid in short exposures causes increased fluidity, in longer exposures it causes coagulation.

It is obvious that our knowledge of the colloid chemistry of protoplasm is extremely fragmentary. The results that have been gained so far indicate, I believe, that a rational beginning has been made.

In the future there are two paths along which further study will develop. In the first place it will become more and more possible to link up the various mechanisms which constitute life with changes in the colloidal condition of the living substance. The living machine, at least in the animal world, is primarily a machine which converts chemical energy into various other types of energy. To explain such a machine some sort of physical explanation or explanations is necessary. No amount of knowledge with regard to the chemical transformations which are involved in life can ever hope to completely solve the riddle as to how the living machine does its work. This is in the ultimate more of a physical than a chemical problem, just as the study of the mechanics of an internal combustion engine is a physical problem. And inasmuch as all living things are built of colloids and little else, it is well nigh obvious that the primary factors which govern life processes are the colloidal changes which occur in the living material. Our biological progress in the future will depend then in large measure on our ability to observe these colloidal changes in the living cell and to correlate them with the various phases in the activity of the living organism.

But this is not the sum total of our problem. If we are able to find that certain changes occur at certain times, and are perhaps indissociably related to the accomplishment of certain vital functions, we are still more at the beginning than at the end of our investigation. We have still to learn what the true nature of these colloidal changes are, what the factors and the forces are which control them. We must seek to find how and in what measure they can be interpreted on the basis of known

or discoverable phenomena in the realm of pure physical chemistry, that is to say the physical chemistry which deals with inanimate chemical compounds of more or less known composition.

This second part of our problem is in many ways the more important. In its investigation it is our hope that the colloid chemist will come to the aid of the biologist.

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University of Michigan,
Ann Arbor, Mich.

THE EFFECT OF SURFACE TENSION DEPRESSANTS UPON BACTERIAL TOXINS

BY W. P. LARSON, H. O. HALVORSON, R. D. EVANS AND R. G. GREEN

During the past few years studies have been conducted in our laboratories on the effect of surface tension depressants upon bacterial growth and bacterial toxins. In this work we have studied the effect of the sodium soaps of various fatty acids upon bacterial growth and bacterial toxins. Early in this work it was found that soaps which tended to form colloidal solutions did not appear to adsorb onto bacteria or their toxins to any great extent. Sodium ricinoleate, however, seems to form true solutions even at relatively high concentrations, and it also has a marked influence upon the growth of bacteria and upon their toxins. The action of this soap upon bacterial toxins has therefore been studied extensively. In previous publications it has been shown that sodium ricinoleate will so affect bacteria that their virulence is lost. It will also change bacterial toxins, without impairing their antigenic properties, so that they no longer produce toxic effects when injected into animals. It has been shown¹ that this surface tension depressant completely destroys the toxicity of tetanus, diphtheria and scarlatinal toxins. In order to do this, however, it has been found that the soap must form a clear solution. The presence of any foreign colloidal particles, even though they be aggregates of other soaps, such as sodium oleate or sodium myristate, prevents the detoxifying action of the sodium ricinoleate. It has been shown that any of the above mentioned toxins may be so completely detoxified by this soap that several hundred fatal doses may be injected into animals without producing any injurious effects. When these detoxified toxins are injected immunity is set up against the normal toxins. Since large doses may be injected immunity is built up quickly. On the basis of these results soap-detoxified toxins have been successfully used in immunizing human beings against diphtheria and against the toxins obtained from the scarlet fever streptococcus.

In carrying out this work it is important to have the proper balance of soap and toxin, so that there will be no injurious effects—not even a local reaction. To this end it is imperative that no foreign colloidal particles be present and that the solutions remain perfectly clear. The

¹ *Proc. Soc. Exp. Biol. and Med.*, 1925, XXII, pp. 550-554.

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presence of mere traces of foreign colloidal particles or precipitates may give rise to severe local and general reactions. It has also been found that it is necessary to allow the mixtures to stand for a short time for the soap and toxin to reach equilibrium.

The results of our experimental work seem to indicate that the effect of the soap upon the toxins is an adsorption phenomenon rather than a chemical reaction. We have explained the action in our own minds by assuming that the soap is adsorbed upon the toxin molecules, thereby changing this surface and also changing their ability to adsorb onto the tissues of the animal, where the injurious effects are produced. The toxins are then probably taken care of in the body as any other foreign colloid, with resultant anti-body formation.

In order to obtain some qualitative, as well as quantitative, data upon this phenomenon, a series of experiments was conducted in which the effect of varying the concentration of both the soap and the toxin was studied.

A series of guinea pigs was inoculated with an L + dose of diphtheria toxin which had been treated with varying concentrations of soap solutions, as shown in the following table. Each guinea pig received 0.4 cc. of the toxin to which had been added one cc. of a 1%, 2%, 3%, etc., of sodium ricinoleate solutions. The final concentrations of toxin and soap are given in the table.

THE EFFECT OF VARYING THE CONCENTRATION OF THE SOAP

Guinea Pig No.	Con. of Toxin, L+ per cc.	Dose, L+	Total Volume Injected, c.c.	Con. of Soap in Per Cent	Result
1.....	.715	1	1.4	.715	Died in 3 days
2.....	"	"	"	1.413	" " 11 "
3.....	"	"	"	2.14	" " 28 "
4.....	"	"	"	2.86	Shown paralysis in 40 days. Lived
5.....	"	"	"	3.57	Lived
6.....	"	"	"	4.28	"
7.....	"	"	"	5.00	"
8.....	"	"	"	5.71	"
9.....	"	"	"	6.42	"

The borderline of protection in this case seems to be somewhere between 2.8% and 3.5% of soap. Injections were repeated several times with these percentages with the same general results. The ones receiving 2.86% soap usually showed paralysis in about 40 days and about half of these died. The occasional exception to the rule may be explained by individual variations of animals.

In another experiment the concentration of the soap was kept con-

stant but the concentration of the toxin was varied. The final concentrations and results are given in the following table.

THE EFFECT OF VARYING THE CONCENTRATION OF THE TOXIN
USING 1 PER CENT SOAP

Guinea Pig No.	Con. of Toxin, L+ per cc.	Dose, L+	Con. of Soap in Per Cent	Total Volume Injected, c.c.	Result
1.....	.715	1	1	1.4	Died in 3 days
2.....	.417	"	"	2.4	" " 3 "
3.....	.264	"	"	3.4	" " 3 "
4.....	.185	"	"	4.4	" " 6 "
5.....	.158	"	"	5.4	Lived
6.....	.135	"	"	6.4	"
7.....	.119	"	"	7.4	"
8.....	.105	"	"	8.4	"
9.....	.096	"	"	9.4	"

This was repeated with a 3% soap solution, the results of which are given in the following table.

THE EFFECT OF VARYING THE CONCENTRATION OF THE TOXIN
USING 3 PER CENT SOAP

Guinea Pig No.	Con. of Toxin, L+ per cc.	Dose, L+	Con. of Soap in Per Cent	Total Volume Injected, c.c.	Result
1.....	1.000	1	3	1	Paralyzed. Died in 12 days
2.....	.333	"	"	3	Lived; no symptoms
3.....	.200	"	"	5	" " "
4.....	.142	"	"	7	" " "

In another experiment the concentration of both the toxin and the soap was varied by diluting the mixture with salt solution. A series of mixtures was prepared containing 0.4 cc. of toxin and 1 cc. of a 4% soap solution. The different guinea pigs were injected with these mixtures after they had been diluted with varying amounts of water. The data are given below.

THE EFFECT OF DILUTION UPON A SOAP TOXIN MIXTURE

Guinea Pig No.	Toxin, cc.	4 Per Cent Soap Sol., cc.	H ₂ O, cc.	Total Vol. Injected, cc.	Con. Tox., L+ per cc.	Con. Soap, Per Cent	Dose, L+	Result
1	.4	1.0	0.00	1.4	.715	2.86	1	Died in 41 days
2	.4	1.0	1.00	2.4	.416	1.66	1	Lived
3	.4	1.0	2.00	3.4	.300	1.18	1	Died in 3 days
4	.4	1.0	3.00	4.4	.227	0.90	1	Died in 2 days

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In the above experiment the soap and toxin were mixed and after 15 minutes' standing the salt solution was added. This experiment has been repeated several times and confirmed.

In another experiment a soap toxin mixture was prepared which would protect the guinea pig. After allowing the mixture to stand for 24 hours a small amount of CaCl_2 was added. The guinea pig which received the soap toxin treated with CaCl_2 died, whereas the one which received the soap and toxin without the CaCl_2 lived. This has been repeated several times with the same result. In this case the CaCl_2 precipitated the soap, thus liberating the toxin.

These series of experiments seem to indicate that the action of the soap is an adsorption phenomenon. It is apparent from our experiments that the concentrations of both the toxin and the soap are of prime importance. The above experiments may be interpreted as follows:

1. A toxic mixture may be rendered non-toxic by diluting the toxin and keeping the concentration of the soap constant.
2. A toxic mixture may be rendered non-toxic by increasing the concentration of the soap without changing the concentration of the toxin.
3. A toxic mixture may be rendered non-toxic merely by adding the proper amount of salt solution to the mixture. This is again changed to a toxic mixture by further dilution.

The above experiments may be explained by assuming that in the free toxin preparations the toxin exists in colloidal aggregates capable of dispersion. In order to completely protect an animal it is necessary to disperse these aggregates and adsorb a layer of soap over the entire surface of the toxin molecule. This dispersion may be brought about either by adding a sufficiently concentrated soap solution to the toxin to bring about the dispersion, or by diluting the toxin. After the aggregates are dispersed toxin is rendered non-toxic by adsorbing a layer of soap around each molecule or molecular aggregate. The mathematical theory underlying this viewpoint has previously been pointed out in a paper by Halvorson and Green presented before the Colloidal Symposium last year.

If this theory is correct, a curve obtained by plotting the concentration of soap necessary to protect various concentrations of toxin against the concentration of toxin would be of the nature of a parabola. In this system it is possible to select such a solution that mere dilution with salt solution will change a nonprotective solution to one that will protect and again to one that will not protect. This is possible since the curve representing the change of concentration of soap and toxin upon dilution would be a straight line. This can be visualized by imag-

ining, in the first place, that the concentration of soap is high enough to affect the adsorption, but not high enough to cause sufficient dispersion. By a small dilution the toxin is completely dispersed, but the concentration of soap is still high enough to affect the adsorption. By further dilution the concentration of the soap is made too low to affect the adsorption.

It is impossible in a limited time and with a limited number of experimental animals to plot an accurate curve of this type. Even

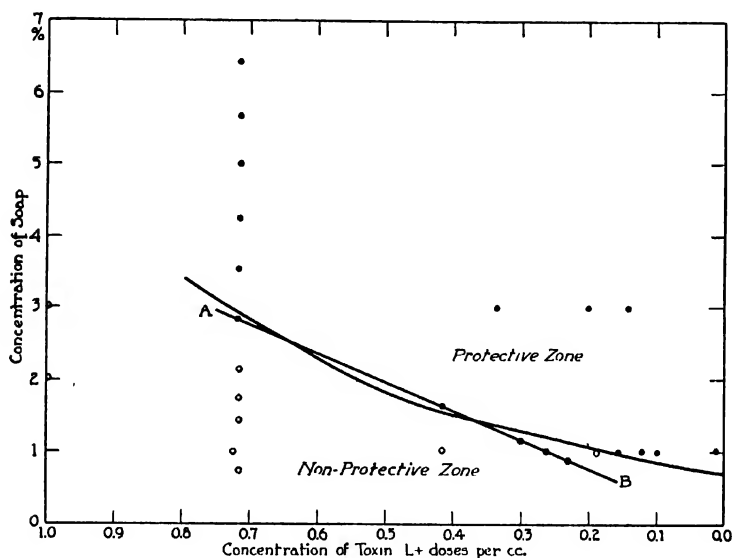


FIG. 1.—The effect of concentration upon the toxicity of soap-toxin mixtures.

though the animals were available, the experiment would necessarily be limited by the volumes which could be injected. In some parts of the curve they would have to be too large and in others too small to be practicable. Furthermore, it is impossible to get toxins of infinite concentrations with which to begin the experiment.

A qualitative confirmation of the curve may be obtained from our data. In this curve the dots represent mixtures which are non-toxic, whereas the circles represent those which are toxic (Fig. 1).

If our viewpoint is correct, the only limit to the dosage of a toxin soap mixture is the rate at which the toxin is liberated in the animal body. If the toxin is poorly protected, this rate will be fast; if it is well protected, this rate will be slow. A very high dosage, however,

could not be protected under any condition because the amount of toxin liberated per unit time would be too great to be overcome by the animal. If the dosage were to be plotted against the concentration of soap required for its protection, an adsorption curve should be obtained. For the same reasons stated above an accurate curve of this type has not as yet been evaluated. Our data confirm this idea, however, in a qualitative way.

Experiments which are in progress at the present time seem to indicate that concentrations are not only of prime importance in the above consideration but that the concentration of the toxin rather than the dosage is the prime factor in controlling the amount of antibody formed. In the immunizing work that has been done with soap toxin mixtures it has been found that with a given concentration of toxin the antibody titre of the animal quickly rises to a certain value above which it will not go, irrespective of the dosage. A higher titre can be obtained only when a higher concentration of toxin is injected.

University of Minnesota,
Minneapolis, Minn.

PHYSICO-CHEMICAL STUDIES OF THE MECHANISM OF BLOOD CLOTTING

BY I. NEWTON KUGELMASS

I. INTRODUCTION

Blood clotting consists essentially in the transformation of soluble fibrinogen into insoluble fibrin. The mechanism in this transition is assumed to be through the action of thrombin. The theories of Morawitz,¹ of Bordet,² of Herzfeld and Klinger,³ of Howell⁴ are concordant in this general view while in the theory of Nolf⁵ thrombin is a product of coagulation and not its cause and in the theory of Hekma⁶ fibrin is the product of neutralization or of dehydration of fibrinogen, the alkalihydrosol of fibrin in the blood stream. The diagrammatic representation of these current theories presents, in the main, interpretation of these concepts.

Analysis of these views reveals a number of fundamental inadequacies incompatible with the great mass of experimental data or the known facts on blood clotting. The prevalence of the alleged hypothetical substances involved in clotting, the lack of evidence of their existence in normal circulating blood, the empiricisms advanced for their behavior, the indefiniteness of the chemical and physical properties of the indispensable components in clotting,—these and many another striking deficiencies in the prevalent theories led us to believe that the best first approximation of an insight into the actual mechanism of blood clotting can only be attained, for the present, by rational systematic physico-chemical studies of the process per se. With the funda-

¹ P. Morawitz, Zur Kenntniss der Vorstufen des Fibrinfermentes, *Hofmeister's Beitr. z. Physiol. u. Pathol.*, 4, 381-420 (1903); Beiträge zur Kenntniss der Blutgerinnung., *ibid.*, 5, 133-141 (1904); *Deuts. Arch. f. Klin. Med.*, 79, 1-28 (1908); 79, 215-233, 432-438 (1904); Zur Frage der Blutgerinnung., *Bioch. Zeits.*, 18, 30-38 (1909).

² J. Bordet, Considérations sur les théories de la coagulation du sang, *Ann. Inst. Pasteur*, 34, 581-595 (1920).

³ E. Herzfeld and R. Klinger, Studien zur Chemie und Physiologie der Blutgerinnung, *Biochem. Zeits.*, 85, 145-188 (1916).

⁴ W. H. Howell, The rôle of antithrombin and thromboplastin (thromboplastic substance) in the coagulation of blood, *Archer Journ. of Physiol.*, 29, 187-209 (1911); The nature and action of the thromboplastic (zymoplastic) substance of the tissue, *ibid.*, 31, 1-12 (1912). "A Textbook on Physiology," 17th edition, Philadelphia and London, 1918, p. 461.

⁵ P. Nolf, Contribution à l'étude de la coagulation du sang, *Arch. Int. Physiol.*, 4, 165-215 (1906); 6, 1-72, 115-191 and 306-359 (1908); quelques faits relatifs à la coagulation du sang, *Bull. Cl. Sciences Acad. roy. de Belgique*, 637-641 (1913).

⁶ E. Hekma, Ueber das Fibrin und seine Beziehung zu einigen Fragen der Biologie einer Kolloidchemie mit besonderer Berücksichtigung des Blutgerinnungs Problems, *Biochem. Zeits.*, 45, 311-331 (1914); 73, 370-453 (1916); 84, 63-92, 219-238 (1916); 77, 249-267, 273-282 (1916).

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mentals of physical and colloidal chemistry intact, its principles must of necessity apply to this—a physico-chemical process.

The experimental studies presented follow kinetically the changes in the physico-chemical properties *during* clotting. Such results reproducible for all clotting systems give a mechanistic expression to the admissible current concepts of clotting and hold no matter what purely chemical researches might reveal in the future.

II. PREPARATION OF THE COMPONENTS OF THE COAGULATION SYSTEM

- (1). *Fibrinogen*—prepared by the method of Dale and Walpole⁷ and by that of Hammerstein modified by Nolf and by Sumner.⁸
- (2). *Oxalated Plasma*—prepared according to the method of Bordet and Delange.⁹
- (3). *Cytozyme or Thromboplastin*—prepared according to the method of Bordet and Delange¹⁰ from rabbit's muscle.
- (4). "Serum" was prepared from very limpid plasma one volume to four of physiologic saline containing Ca ions. After clotting the fibrin, the solution was filtered and allowed to stand two days.
- (5). *Physiologic Saline* (P. S.) 6 gms. of NaCl per liter of water.
- (6). *Physiologic Saline* containing Ca ions (P. S. Ca) 6 gms. of NaCl and 35 centigrams of CaCl₂ per liter of water.

Preliminary experiments were made so regulating the concentration factor of the clotting components that coagulation was realized in about thirty minutes in order to obtain duplicable results for each of the experiments per se and thus have the time range as one of the constant factors so that authentic comparisons among the various physico-chemical properties may be made.

Each clotting system contained 0.3 cc. P. S. Ca, 0.5 cc. of a suspension of cytozyme, 0.2 cc. "Serum" and 0.5 cc. P. S. After one-half hour 0.5 cc. oxalated plasma or fibrinogen was added.

Each of the physico-chemical experiments were repeated unto duplicable satisfaction. The results cited are representative of such series and are relative indicating the trend of change during coagulation. Suffice it to say that such changes in the magnitudes of the properties during

⁷ H. H. Dale and G. S. Walpole, Some experiments on factors concerned in the formation of thrombin, *The Biochem. Journ.*, 10, 831-862 (1916).

⁸ J. B. Sumner, A propos de la purification des solutions de fibrinogene et de l'adsorption du cytozyme, du sérozyme et de la thrombine, *C. R. Soc. Biol.*, 87, 888-891 (1922).

⁹ J. Bordet and L. Delange, La coagulation du sang et la formation de la thrombine, *Ann. Inst. Pasteur*, 26, 657-674, 737-766 (1912).

¹⁰ J. Bordet and L. Delange, Sur la nature du cytozyme, recherches sur la coagulation du sang, *Ann. Inst. Pasteur*, 27, 841-857 (1913).

clotting offer a sound basis for the interpretation of the dynamic physico-chemical mechanisms involved.

The experimental development of these studies is as follows:

1. The rôle of the hydrogen ion concentration in clotting; the isoelectric points of the blood proteins; the cII limits for clotting.
2. The changes in conductivity during clotting; the modifications in the ionic concentrations during clotting.
3. The changes in the protective power of the proteins.
4. The relations between component concentrations of the clotting system on the rate of coagulation, the temperature coefficient of the clotting reaction.
5. The changes in viscosity in the course of coagulation; the modifications of the degree of transparency of the clotting system during coagulation.
6. The changes in the surface tension of the clotting system during coagulation.

III. RÔLE OF THE HYDROGEN ION CONCENTRATION IN BLOOD CLOTTING

Researches on blood clotting have to date ignored the hydrogen ion concentration factor in spite of its extreme sensitivity in effecting marked changes with its variations on the properties of proteins as the works of Hardy, Pauli, Michaelis and Loeb¹¹ have demonstrated.

All hydrogen ion experiments were carried out potentiometrically at 40 mm. CO₂ tension, with the necessary precautions developed by Michaelis, Cullen, Clark¹² and others.

Changes in the Hydrogen Ion Concentration During Clotting

At first the cH of each of the clotting components was measured (Table I, A) so as to offer a basis for comparison of the changes brought about during clotting. Once the initial equilibrium cII value was determined the changes in the cH were followed at definite time intervals (Tables I, B and C).

The variations in the hydrogen ion concentration during clotting represent a continuous phenomenon. The curves representing the rates of diminution in hydrogen ion concentration are similar for the systems: plasma-thrombin and fibrinogen-thrombin. It will be further observed that the rate of diminution of hydrogen ion concentration is great at the first intervals but that it gradually falls off approaching zero, i.e., an asymptotic limit. Comparing the initial cH values of plasma or

¹¹ J. Loeb, *Amphoteric Colloids, Journ. of Gen. Physiol.*, **1**, 89-60, 237-254 (1918).
"Proteins and the Theory of Colloidal Behavior," New York, 1922.

¹² W. M. Clark, "The Determination of Hydrogen Ion," Baltimore, 1922.

THEORIES ON BLOOD CLOTTING

I	{ Schmidt Morawitz 1865 1904	(Platelets)	\rightleftharpoons [Thrombogen]	[Ca ⁺⁺]	\rightleftharpoons [Thrombin]	\rightarrow [Fibrin]	
		(Cellular Elements)	\rightleftharpoons [Thrombokinas]	[Antithrombin]	\rightarrow [Thrombin]	\rightarrow [Fibrin]	
		Fuld and Spiro 1904			[Fibrinogen]		
II	{ Bordet 1912	(Serum)	\rightleftharpoons [Proserozyme]	Contact Catalysis \rightarrow [Serozyme] (Thermolabile)	[Ca ⁺⁺]	\rightarrow [Thrombin]	\rightarrow [Fibrin]
		(Platelets Leucocytes Tissue Juices)	\rightleftharpoons	\rightarrow [Cytzyme] (Thermostable)		[Fibrinogen]	
		Herzfeld and Klinger 1916					
III	{ Woolbridge Nolf 1883 1908	[Fibrinogen]	\rightleftharpoons	Thromboplastics [Ca ⁺⁺] (Contact, H ₂ O, CHCl ₃ , lipoids etc.)		1. [Fibrinogen-Thrombogen- Thrombozyme]	= Fibrin
		[Thrombogen]	\rightleftharpoons			2. [Thrombogen- Thrombozyme]	= Thrombin
		[Thrombozyme]	\rightleftharpoons	[Antithrombin]		3. [Thrombogen]; - [Thrombozyme]	= Excess components
IV	Howell 1910	[Prothrombin]	\rightleftharpoons	[Ca ⁺⁺]	\rightarrow [Thrombin]	\rightarrow [Thrombin-Fibrinogen]	= Fibrin
		Antiprothrombin	\rightleftharpoons		[Fibrinogen]	Antithrombin	
						(Platelets Leucocytes Tissue Cells)	\rightleftharpoons [Thromboplastin]
V	Hekma 1915	[Fibrin alkali hydrosol] (hydrophile)	\rightleftharpoons	Dehydration Neutralization [OH] ⁻	\rightarrow [Fibrin Sol] (hydrophobe)	\rightleftharpoons [Fibrinogel] (hydrogel)	
						Hydroxions Hydration	

TABLE I

(A)

Components at 26° C. 762 mm.	E.M.F.	pH	cH
P.S.	0.715	6.5	0.32×10^{-8}
P.S. Ca.	0.710	6.4	0.40×10^{-8}
"Serum"	0.740	6.8	0.18×10^{-8}
Cytozyme	0.730	6.6	0.25×10^{-8}
Thrombin soln.	0.700	6.2	0.63×10^{-8}
Oxalated plasma	0.770	7.5	0.32×10^{-7}

(B)

CHANGES IN THE HYDROGEN ION CONCENTRATION DURING THE COAGULATION OF PLASMA

Time in Minutes After Mixing	E.M.F.	pH	cH
0	0.710	6.50	0.32×10^{-8}
15	0.725	6.70	0.20
25	0.735	6.82	0.16
40	0.738	6.90	0.13
55	0.740	6.97	0.11
75	0.741	7.00	1.00×10^{-7}
90	0.743	7.02	0.95

(C)

CHANGES IN THE HYDROGEN ION CONCENTRATION DURING THE COAGULATION OF FIBRINOGEN

Time in Minutes After Mixing	E.M.F.	pH	cH
Fibrinogen and Thrombin solution	0.760	7.30	0.50×10^{-7}
	0.710	6.40	0.40×10^{-8}
0	0.722	6.64	0.23×10^{-8}
20	0.728	6.76	0.17
40	0.737	6.85	0.14
60	0.738	6.90	0.13
80	0.739	6.92	0.12

fibrinogen with those of the system containing fibrin as a component at equilibrium after coagulation, it is at once evident that the initial values of plasma or fibrinogen were at a smaller cH (*i.e.*, more on the alkaline side), while the resultant fibrin is at a higher cH (or more on the acid side). The fibrinogen then does not lose OH ions per se but adsorbs H in the process of coagulation. The results are in accord with those of Hekma¹⁸ in that the resultant fibrin is less alkaline than its

¹⁸ E. Hekma, Fibrine in gel and sol condities Bloedstemming, *K. Akad. v. Wetenschappen te Amsterdam Ver Vergaden. Wissen Natuurk Afd.*, 21, 1449-1465 (1918).

initial fibrinogen. Examining the initial cH value of the system after mixing with that of final cH value after coagulation, we are convinced that there is a diminution in cH. This indicates that the fibrinogen adsorbs H^+ rather than loses OH^- . While the end result is the same the mechanism is not that ordinarily known.

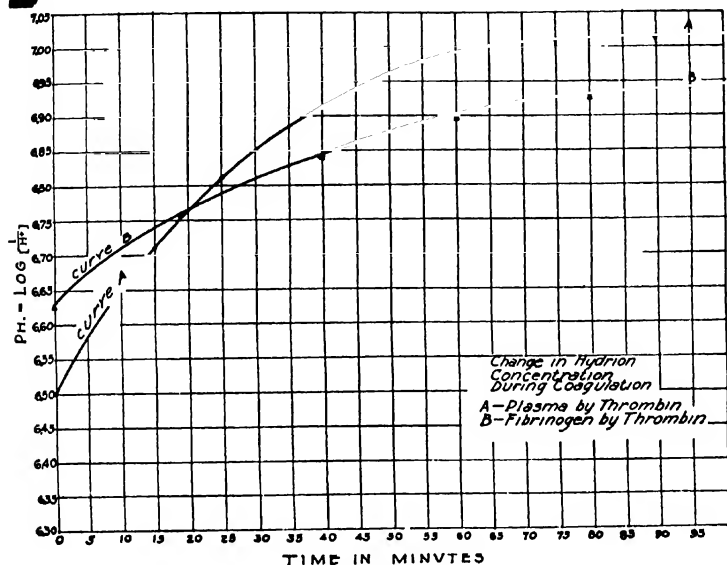


FIG. 1.

These results establish conclusively that fibrin formed as an amphoteric protein has a hydrogen ion concentration lower than the initial cH of the mixture of all components necessary and sufficient for clotting. This fundamental fact puts to serious question all previous comparisons of the initial and final components and their properties since such studies were made at two distinctly different hydrogen ion concentrations, and therefore incomparable.

—B—

Changes in the Hydrogen Ion Concentration Produced by Coagulation in Media of Different Initial Hydrogen Ion Concentration

Having established that a continuous change in the cH is brought about during clotting, the question arose whether this change is a con-

stant irrespective of the initial cH. Solutions of thrombin at various hydrogen ion concentration were prepared by adding calculated quantities of decinormal NaOH or HCl diluted with P. S., and the cH values were measured before and after clotting.

There are apparent limitations to this procedure in that the sole influence of the hydrogen ions is not absolutely realized in non-buffer systems but clotting necessitates the presence of other ions so that the data obtained are indicative of the relative but not absolute magnitudes of change.

TABLE II
CHANGES IN THE HYDROGEN ION CONCENTRATION PRODUCED BY COAGULATION IN MEDIA OF DIFFERENT INITIAL HYDROGEN ION CONCENTRATION

Coagulating System	E.M.F.	pH	cH	Diminution in cH	Percentage Diminution in cH
Initially	0.705	6.3	5×10^{-7}	2×10^{-7}	c. 40
After clotting	0.712	6.5	3×10^{-7}		
Initially	0.645	5.3	3×10^{-8}	1.5×10^{-8}	c. 50
After clotting	0.670	5.8	1.5×10^{-8}		
Initially	0.636	5.15	6.8×10^{-8}	3.8×10^{-8}	c. 56
After clotting	0.655	5.50	3×10^{-8}		
Initially	0.605	4.6	2.5×10^{-8}	1.5×10^{-8}	c. 60
After clotting	0.628	5.1	1×10^{-8}		

From these results it follows at once that:

- There is always a diminution in the hydrogen ion concentration no matter what may be the initial hydrogen ion concentration of the system.
- The higher the initial cH the greater the diminution in hydrogen ion concentration.
- The average value of hydrogen ions disappearing from the coagulating system is $\pm 50\%$.

Limits and Optimum of pH for Coagulation

In the course of the above experiments it was observed that no clotting took place at pH's greater than about 4.9. This suggested a study of the pH limits for clotting preparing the varying cH systems as above. The results (Table III) show clearly that the hydrogen ion concentration markedly affects the rate of coagulation. In particular the following is clear cut:

- Clotting takes place only between pH 5 and pH 8, at least within 24 hrs. at 38° C.

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TABLE III
COAGULATION TIME FOR SYSTEMS OF DIFFERED INITIAL HYDROGEN ION
CONCENTRATIONS

Experiment I at 21° C.		Experiment II at 38° C.	
pH	Clotting Time	pH	Clotting Time
4.9	0 after 24 hrs.	4.5	0 after 24 hrs.
5.1	1.20 minutes	5.0	155 minutes
5.3	96 "	5.5	113 "
5.5	70 "	6.0	65 "
5.8	55 "	6.5	59 "
5.3	44 "	7.0	40 "
7.0	38 "	7.5	68 "
7.4	50 "	8.0	95 "
7.9	80 "	8.5	0 after 24 hrs.
8.0	140 "		
8.2	0 after 24 hrs.		

- (2) On the acid side of neutrality, the greater the cH the slower the clot formation.
- (3) On the alkaline side of neutrality the greater the OH concentration the slower the clotting.
- (4) The hydroxions retard clotting to a much greater extent than the hydrions.
- (5) The optimum for the rapidity of clotting is around pH 7.
- (6) On the acid side of neutrality, as the hydrogen ion concentration is increased the continuity of the resultant reticulum becomes less marked until at higher cH's a distinct cleavage of the reticulum occurs, yielding separate floccula up to pH 5.
- (7) On the alkaline side of neutrality, as the cOH increases disappearance of the visible reticulum occurs yielding very fine structured fibrin of very soft consistency until pH 8 when fibres so diminish in breadth and number that they pass out of the range of ultramicroscopic visibility.
- (8) The anticoagulant action of the high cH on the one side and the relatively high cOH on the other is not upon the thrombin since neutralization of such thrombin solution yields a clot. Media of rather high cH and cOH (as defined by the limits of clotting) evidently prevent the formation of fibrin.

IV. ISOELECTRIC POINTS OF THE BLOOD PROTEINS

Clotting limits so distinctly defined between pH 5 and pH 8 with optimum at pH 7, it appeared reasonable to assume that these cH values must of necessity be related to protein properties characterized by hydrogen ion concentrations, *i.e.*, their isoelectric points.

The Isoelectric Point of a protein is defined as that reaction where the ratio of the hydron concentration to the hydroxion concentration in the solutions is the same as the ratio of the acid dissociation constant (K_a) of the protein to its basic dissociation constant (K_b). When the dissociation constants of a simple amphoteric electrolyte or ampholyte protein are known, the isoelectric point I may be calculated from the relation $I = \sqrt{\frac{K_a K_b}{K_w}}$ where $K_w = (H^+)(OH^-) = 10^{-14}$. When $K_a = K_b$ the isoelectric point will correspond with 10^{-7} or neutrality, if $K_a > K_b$ the isoelectric point shifts to the acid side. If $K_a < K_b$ the point shifts to the alkaline side. In other words, if we add a protein to a solution whose hydron concentration is greater than its isoelectric point, it functions as a base and therefore decreases the hydron concentration of the solution. If we add a protein to a solution whose hydron concentration is smaller than its isoelectric point it functions as an acid and therefore increases the hydron concentration. If we add a protein to a solution whose hydron concentration is not altered, then the hydron concentration of the solution must equal the isoelectric point of the protein. At the isoelectric point the number of protein anions equals the number of protein cations present and the total protein ions in proportion to the molecular protein is at its minimum. This is the concept initiated by Hardy¹⁴ and developed by Michaelis¹⁵, Loeb¹¹⁻¹⁶ and Pauli.¹⁷

The proteins studied thus far have proven to be most inert at their isoelectric points. As the hydron concentration is increased the electro-positive or cationic activity of the proteins is increased. As the hydroxion concentration of the solution is increased the electronegative or anionic activity of the same proteins is likewise increased. This distinctly physico-chemical amphoteric behavior of the proteins and the consequent changes in properties effected fundamentally by change in cH in the system, must be considered wherever protein reactivity is involved.

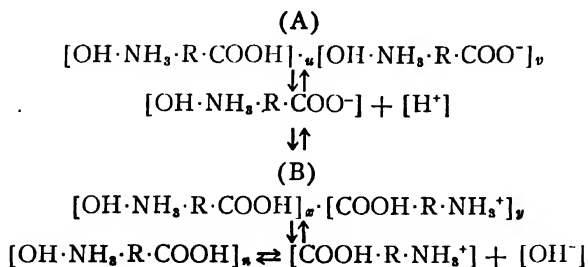
The chemical reactivity of the proteins for ionogens in solution is due to their available amido and carboxyl groups. These are considerably increased by the protein micellar aggregation. A water solution of a protein may be represented by the following equilibria:

¹⁴ W. B. Hardy, Eine Vorläufige Untersuchung der Bedingungen welche die Stabilität von nicht umkehrbaren Hydrosolen bestimmen, *Zeits. f. physical Chem.*, 33, 385-400 (1900).

¹⁵ L. Michaelis and B. Mostynski, Die isoelectrische Konstante und die relative Aciditätskonstante des serumalbumines, *Biochem. Zeits.*, 24, 79-91 (1910); L. Michaelis and P. Rona, Beiträge zur allgemeinen Eiweisschemie. I. Die Koagulation des denaturierten Albumins als Funktion der Wasserstoffionenkonzentration und der Salze, *ibid.*, 27, 38, 52 (1910).

¹⁶ J. Loeb, The possible influence of the amphoteric reaction of certain colloids upon the sign of their electrical change in the presence of acid and alkalis, *Univ. of Calif. Publ. Physiology*, 1, 149-150 (1904); *J. Gen. Physiol.*, 1918, 1919, 1920, 1921, 1922.

¹⁷ Wolfgang Pauli, "Colloid Chemistry of the Proteins," 1922, Philadelphia.



If concentration (A) is greatest as in serum albumin, addition of hydrions with a buffer solution shifts the equilibria from A to B until concentration (A) = (B) at the isoelectric point. At this reaction proteins display a general behavior as do most ampholytes.

Conditions of an Ampholyte at Its Isoelectric Point

- (a) Properties which present their minimum at the isoelectric point.
 1. Viscosity.
 2. Osmotic pressure.
 3. Swelling.
 4. Conductivity.
 5. Solubility.
 6. Alcohol Index.
 7. Optical rotation.
- (b) Properties which practically disappear at the isoelectric point.
 8. The speed of micellae per unit potential.
 9. Electric charge.
- (c) Properties which attain equality at the isoelectric point.
 10. Concentrations of ions and cations.
 11. Migration number of ions toward anode and cathode.
- (d) Properties which present their maximum at the isoelectric point.
 12. Flocculation.
 13. Opalescence.
 14. Degree of non-dissociation of the ampholyte.
 15. Speed and quantity of crystallization.

Determination of the Isoelectric Points of Proteins

On the basis of the researches of Michaelis and Loeb demonstrating the minimum activity of proteins at the isoelectric point, the experimental methods used were based on the *determination of the point of inflection of some of the properties of the proteins with change in hydrion*

concentration in the solution system. The necessary and sufficient criterion to obtain the *sole* influence of the hydron concentration in a system on its containing protein was to maintain constant all other ions in the system. Such solutions were made up of mixtures of acid with its salt termed "buffer solutions" which produced a stabilized hydron concentration. Strong acids cannot be used for a hydron source directly because they yield an isoelectric point at a smaller cH. This is due to salt formation of the strong acid in the isoelectric region.

Preparation of the Proteins of Blood Plasma

Fibrinogen was prepared by the method of Dale and Walpole.⁷ The solution on addition of a few drops of toluol was dialyzed for several days until the conductivity had become less than 1×10^{-5} megohms. The fibrinogen is now extracted with alcohol, dried and pulverized.

Fibrin was obtained by adding four volumes of P. S. Ca concentration to one volume of exhaled plasma from rabbit's blood. After gelation of the mass the fibrin is separated by glass rod and washed free from salts, and finally washed with alcohol and dried.

Seroglobulin was prepared by adding to the serum one volume of saturated aq. ammonium sulfate. The precipitate is centrifuged and redissolved in water, reprecipitated with an equal volume of saturated ammonium sulfate and these precipitations repeated several times and dialyzed as in the case of fibrinogen.

Seroalbumin is obtained by saturating the supernatant liquid from the previous centrifugations by means of finely pulverized ammonium sulfate. The albumin precipitate is separated, redissolved in water. This is repeated several times and dialyzed as above.

Methods of Procedure

- (a) *Turbidity Method*—Equal quantities of the pure prepared protein solution were added to the standard pH buffer series and the tube in which the turbidity optimum appeared defined the pH range of the isoelectric point. Final determination of this value were obtained by another pH series within the limits just found. Shades of intensity were measured by a devised photo-electric arrangement described in another paper.¹⁸ This was applied to serum albumin, globulin and fibrinogen.
- (b) *Flocculation Method*—Same as in (a). The optimum flocculation corresponding to the isoelectric point as applied to fibrinogen and serum globulin.

¹⁸ I. Newton Kugelmass, Un nouvel appareil: le Nephelectrometre, *C. R.*, **175**, 843 (1922).

- (c) *Alcohol Number*—is the number of cc. of 95% alcohol necessary to precipitate one cc. of the prepared protein solution. Genuine serum albumin as an isostable substance is not flocculated at its isoelectric point unless dehydrating compounds like alcohol are added.
- (d) *Dye Method*—Equal portions of powdered globulin and fibrin were placed in pH series for two hours, filtered, washed with distilled water and 0.001% solutions of acid dye Eosin in one

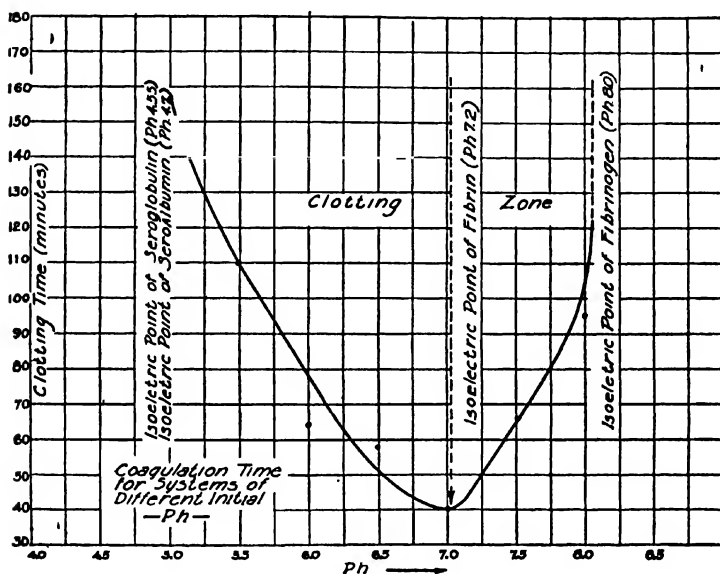


FIG. 2.

series and a 0.001% solution of basic dye Fuchsin in another series were then poured through. Protein will combine with the colored cation of the basic dye when on the alkaline side of the isoelectric point and with the colored anion of the acid dye when on the acid side of the isoelectric point. Those samples which showed minimum color fixation were recorded as isoelectric. Potentiometric measurements of the hydron concentration of the solutions were made for those samples that were judged by the above methods to be in the isoelectric condition. (See Table IV and Fig. 2.)

These studies indicate that the clotting limits are defined by the isoelectric points of serum albumin (pH 4.7) and globulin (pH 4.5) on

TABLE IV

Protein	Method Employed	Isoelectric Points		
		Values Obtained	Average	pH
Seroglobulin	Turbidity Flocculation	3.4×10^{-8} 2.6×10^{-8}	3×10^{-8}	4.55
Seroalbumin	Turbidity Flocculation	1.5×10^{-8} 2.5×10^{-8}	2×10^{-8}	4.7
Fibrin	Dye Swelling	0.25×10^{-7} 0.35×10^{-7}	0.3×10^{-7}	7.2
Fibrinogen	Turbidity Dye	1.4×10^{-8} 0.6×10^{-8}	1×10^{-8}	8.0

the one hand and of fibrinogen (pH 8) on the other and that of fibrin (pH 7.2) is equivalent with the optimum pH for clotting.

This offers so clear and convincing a picture that at once it is evident that the conditions necessary and sufficient for the coagulation of the blood are:

- (a) That the serum proteins be on the anionic side of their isoelectric points, *i.e.*, that they may behave as hydrophil and emulsoids.
- (b) That the fibrinogen be on the cationic side of this isoelectric point, *i.e.*, that it may behave as a hydrophobe suspensoid.
- (c) That the fibrin be at its isoelectric point for most rapid rate of formation and for maximum yield and maximum elasticity.

V. MODIFICATIONS OF THE IONIC CONCENTRATION DURING COAGULATION

A. Measure of the Conductivity During Clotting

I

Changes were observed in the hydron concentration during blood clotting so it became of interest to make corresponding measurements of conductivity. Solutions of thrombin and plasma were prepared by the usual technique. Measurements of the electrical resistance of the mixture were made at definite time intervals during coagulation and compared with a standard electrical resistance. The direct method of meas-

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TABLE V
(A)

Components of the Clotting System	Conductivity in Megohms
Oxalated plasma	27.8×10^{-3}
Serum exuded from plasma.....	30.0×10^{-3}
P.S.	38.0×10^{-3}
P.S. Ca.	75.0×10^{-3}
Solum thrombin	64.0×10^{-3}

(B)

Conductivity After	Conductivity Megohms
0 Minutes	65.8×10^{-3}
10 "	58.4×10^{-3}
20 "	54.5×10^{-3}
30 "	52.1×10^{-3}
35 " —Clot complete	
40 "	51.8×10^{-3}
50 "	50.4×10^{-3}
60 " Liquid exuded "Serum".....	50.3×10^{-3}
70 "	50.2×10^{-3}

uring electrical resistance using the triple electrode lamp was employed.¹⁹ Results obtained are given in Table V.

The type of relation which was found to subsist between the time which elapsed after the mixing of all components necessary for clotting and the resultant changes in conductivity is shown in data above. It will be observed that the rate of diminution in conductivity is at first great but that it rapidly falls off appearing to approach an asymptote—the limit defining final equilibrium. If the product of the action of the thrombin on fibrinogen were a true gel there would be little change in the electrical conductivity during its formation as the resistance of a gel to the passage of ions is practically the same as that of the sol from which it has been formed. The change in conductivity during clotting indicates that more than a physical modification has taken place, the exact nature of which may be revealed by further studies in progress. Specifically, on comparing the ordinates revealing the decrease in conductivity with those showing decrease in hydrogen ion concentration, it appears that the conductivity decrease is more marked than would be expected if it were solely due to a loss in hydrogen ions during clotting. To determine what other ions disappear as such in the course of clotting, the studies presented below were carried out with that view in mind.

¹⁹ M. Philippon, *Arch. int. Physiol.*, 18, 161-172 (1921).

II

The presence of fibrin in its filamented spongy network must increase the conductivity of the solution to some extent depending on amount of and nature of the ions that are condensed, on its surface or dispersed within its meshes. The clot was then filtered, the volume of the solution recorded, and the clot washed with hot conductivity water several times. The conductivity of the clot filtrate and washings was measured after concentration to its initial volume. (Table VI.)

TABLE VI
CHANGES IN CONDUCTIVITY AND HYDRION CONCENTRATION EFFECTED BY
PRESENCE OF CLOT

In Mhos. $\times 10^{-8}$		Hydrion Conc.	
Before	After	Before	After
51.2	56.5	0.60×10^{-7}	1.30×10^{-7}

The data above show an increase in the conductivity and in the hydrion concentration but not to the original values. The extent of experimental error is not that great, as fairly duplicate results are obtainable. It is clearly evident that hydrions are bound by the fibrin. To what extent other ions are adsorbed will be the subject of further study by alkali metal potentiometric chain method, development of which is in progress.

III

To determine the relative effects of sodium and calcium salts on the changes in conductivity during coagulation the solutions were prepared as follows:

A. Fibrinogen	9.0 cc.	C. E/20 NaCl	1.5
Cytzyme	1.2 cc.	D. E/20 CaCl_2	1.5
Serum	3.2 cc.	E. P. S.	2.5 cc.
B. P. S.	3.0 cc.	P.S.Ca	2.0 cc.
P.S.Ca	3.0 cc.		

The following mixtures were prepared from these five solutions.

- (1) $\frac{1}{3}$ A + $\frac{1}{2}$ B + C (Na system).
- (2) $\frac{1}{3}$ A + $\frac{1}{2}$ B + D (Ca system).
- (3) $\frac{1}{3}$ A + E (Normal system).

The results of the changes in conductivity in these three mixtures prepared for clotting are given below. For brevity that solution which contains an excess of calcium ions is referred to as the (Ca system);

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that containing an excess of sodium is referred to as the (Na system), and the third as the (Normal system).

TABLE VII

Time	Na System	Ca System	Normal System
0 minutes.....	72.0	70.0	65.8
10 "	66.2	62.8	58.4
20 "	62.0	58.1	54.5
30 "	—	54.0	52.1
35 "	—	—	— (clot)
40 "	56.2	51.0	51.8
50 "	54.0	—	50.4
60 "	— (clot)	48.8 (clot)	50.3
70 "	53.0	48.0	50.2

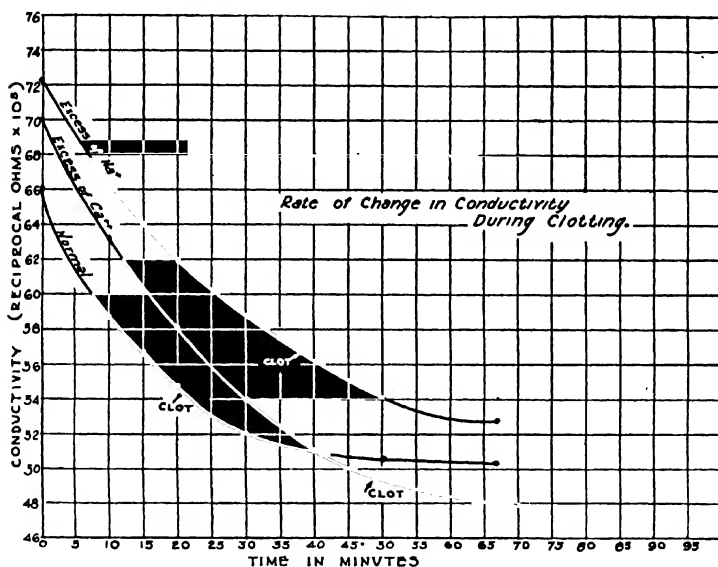


FIG. 3.

A comparison of the conductivity behavior during coagulation of the three systems (*vide* Curve) reveals some striking differences.

- (a) That initially the conductivity of (Na system) > (Ca system) > (Normal system)
- (b) That the conductivities decrease to a greater extent in the calcium system than in the sodium system

- (c) That during actual clot formation the calcium system has the minimum conductivity while the sodium system has the maximum conductivity
- (d) That calcium excess delays clot formation
- (e) That the elasticity of the clot in (Na system) > (Normal system) > (Ca system)
- (f) That syneresis in the (Na system) > (Normal system) > (Ca system)

Interpretation of the rôles of sodium and calcium salts in clotting may be developed on a colloid-chemical basis. The components of each reacting system are: water, proteins, lipoids and salts—in hydrophylic emulsoid condition. Clowes²⁰ has shown that sodium oleate emulsifies oil in water and calcium oleate emulsifies water in oil and a mixture of the oleates behave differently depending on the relative amounts present. In the oil-in-water emulsion conductivity should increase whereas in the water-in-oil emulsion conductivity should decrease. In our system it appears that the sodium lipid increases the degree of emulsification of serum proteins in water and the calcium lipid emulsifies salt solution in the fibrin when formed. The fibrin network seems to consist of salt solution emulsified in the fibrin meshes while the fluid contained in the interstices or the intermicellar fluid (Duclaux) is a saline emulsion of serum proteins. Equilibrium between the two emulsion systems is gradually approached. The more emulsified saline water in the fibrin reticulum of meshes the less the spontaneous contraction or syneresis of the clot.

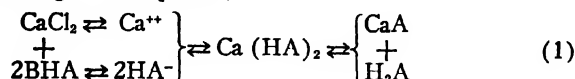
The Buffer Mechanism for the Calcion Concentration and the Determination of Calcion Buffer Values

An aqueous saturated solution of calcium chloride has a calcion concentration of about 5 mols per liter at 38°. A neutral saturated solution containing carbonates and phosphates at 38° has a calcion concentration of 0.0025 mol per liter (1). The calcion concentration of the first solution is two thousand times that of the second solution. These two solutions are analogous to an unbuffered and a buffered system of hydrions. The second solution is a buffered system in which additions of calcium salts do not increase the calcion concentration proportionally to the amount added, nor does the removal of calcium salts decrease it proportionally. The calcion concentration is stabilized as the hydrion concentration is stabilized in buffered systems. The relations which determine the calcion concentration are the subject of this study.

²⁰ C. H. A. Clowes, *Protoplasmic Equilibrium*, *J. Phys. Chem.*, **20**, 407-451 (1916); Bancroft, *Applied Colloid Chem.*, 267 (1921).

The Nature of Calcion Buffers

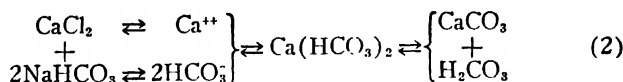
Addition of a highly dissociated calcium salt, *e.g.* CaCl_2 , produces a reaction in a mixture of a weak buffer acid, H_2A , and its primary salt, BHA, according to the equation,



The highly dissociated calcium salt combines with the buffer salt, BHA, to form the intermediate salt, $\text{Ca}(\text{HA})_2$. This calcium buffer salt is always in equilibrium with the least soluble, normal salt, CaA. The equilibrium is determined by the concentrations of buffer salt, BHA, and buffer acid, H_2A . Increase of concentration of calcium or buffer salt shifts the equilibrium from left to right; increase of concentration of buffer acid, from right to left. The calcion concentration is decreased in the first instance and is increased in the second instance. At equilibrium, calcion concentration is determined by the relative concentrations of total buffer salt, BHA plus $\text{Ca}(\text{HA})_2$, and free acid, H_2A . This constitutes a calcion buffer mechanism comparable to that for the hydrion buffer mechanism.

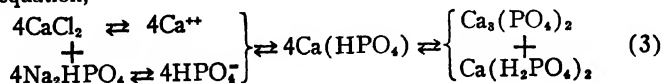
Calcion buffers are substances which resist the change in calcion concentration upon addition or removal of calcium salts. The calcion concentration is stabilized in the presence of mixtures of weak acids and their salts, which react to form insoluble normal calcium salts and soluble intermediate calcium salts.

The Carbonates as Calcion Buffers.—As an example of calcion buffering, the carbonates may be chosen. They react with a highly dissociated calcium salt, *e.g.*, CaCl_2 , according to the equation,



where NaHCO_3 and H_2CO_3 are the buffer salt and acid, respectively; $\text{Ca}(\text{HCO}_3)_2$, the intermediate primary calcium salt; and CaCO_3 , the insoluble normal calcium salt. The calcion concentration of this system at equilibrium is determined by the ratio of the dissolved $\text{Ca}(\text{HCO}_3)_2$ and CaCO_3 , which is fixed by the ratio of concentrations of total buffer salt, NaHCO_3 plus $\text{Ca}(\text{HCO}_3)_2$, and free buffer acid, H_2CO_3 .

The Phosphates as Calcion Buffers.—The phosphates react in a similar manner with a highly dissociated calcium salt, *e.g.* CaCl_2 , according to the equation,



where Na_2HPO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ are the buffer salt and buffer acid, respectively; CaHPO_4 , the intermediate secondary calcium salt; and $\text{Ca}_3(\text{PO}_4)_2$, the insoluble, normal calcium salt.

The Relations of Calcion Concentration in Terms of Calcion Buffers

The Calcion Concentration in Terms of the Carbonates.—Experimental studies of the equilibrium relations of the carbonates in aqueous solution (1) have shown that at 38° , in the presence of solid CaCO_3 ,

$$\frac{[\text{Ca}^{++}][\text{HCO}_3^-]^2}{[\text{H}_2\text{CO}_3]} = K_1 = 4.1 \times 10^{-5} \quad (4)$$

The HCO_3^- from carbonic acid in the concentration of carbonates equivalent to that present in blood is extremely small; i.e., to the extent of about 1:10,000 of the H_2CO_3 . Essentially all the anion originates from the dissociation of the bicarbonates. Considering any soluble, monovalent, highly dissociable salt, BHCO_3 , as the source of HCO_3^- and assuming its average degree of ionizations to be γ , Equation 2 may be rewritten as

$$\text{Ca}^{++} = K_1 \frac{[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]^2} = \frac{K_1}{\gamma^2} \cdot \frac{[\text{H}_2\text{CO}_3]}{[\text{BHCO}_3]^2} = K' \frac{[\text{H}_2\text{CO}_3]}{[\text{BHCO}_3]^2} \quad (5)$$

Inverting and expressing in logarithmic form,

$$\text{pCa} = \text{pK}' + \log \frac{[\text{BHCO}_3]^2}{[\text{H}_2\text{CO}_3]} \quad (6)$$

Where pCa and pK' are the negative logarithms of $[\text{Ca}^{++}]$ and K' respectively.

The logarithmic unit, pCa , similar to the symbol pH , has been adopted for the numerical expression of the calcion concentration because it is more practical, more convenient to handle mathematically, and because changes in pCa are directly proportional to changes in pH . The physiological significance of this unit cannot be demonstrated because adequate data on the calcion concentration are not yet available.

Equation 6 has been tested against the experimental data given in Table VIII. Calculated from the relation,

$$\text{pK}' = \log \frac{K'}{(\gamma)^2}$$

where K' is 4.1×10^{-5} at 38° , and the average γ of BHCO_3 is 0.85, the value of the calcion pK' for the carbonates is 4.2.

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TABLE VIII
THE EVALUATION OF THE CARBONATES AS CALCIUM BUFFERS

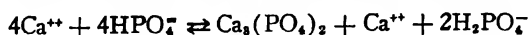
BHCO ₃	H ₂ CO ₃	pCa Calculated from:	
		Determinations	Equation 6
millimols/l	millimols/l		
7.8	2.78	2.48	2.50
8.2	2.78	2.52	2.52
6.6	2.08	2.55	2.57
6.2	1.36	2.60	2.65
5.8	1.36	2.61	2.59
4.6	0.68	2.70	2.69
10.9	2.08	2.82	2.90
9.78	1.36	3.00	3.05
16.38	2.78	3.24	3.19
16.20	2.08	3.30	3.30
15.70	1.36	3.52	3.48
15.48	0.68	3.72	3.75

The calcion buffering of the carbonates expressed by Equation 6 may be illustrated by two comparable experiments. Two aqueous systems at 38° contained in mols per liter:

Experiment	CO ₂ Tension,	H ₂ CO ₃	HCO ₃ ⁻	Ca ⁺⁺	pCa
	mm.				
1	20	0.00068	0.0038	0.00190	2.72
2	20	0.00068	0.0121	0.00019	3.72

Addition of 0.015 M NaHCO₃ to the calcium bicarbonate solution in Experiment 1 increased the HCO₃⁻ 3.1 times. The effect of this bicarbonate increase was a 10-fold decrease in the calcion concentration or a change in pCa from 2.72 to 3.72.

The Calcion Concentration in Terms of the Phosphates.—The relation for the calcion concentration in terms of the phosphates as calcion buffers may be developed similarly to the carbonates. The right-hand member of the buffering reaction 4, expressed ionically is,



The law of mass action gives,

$$\frac{[\text{Ca}^{++}]^4 [\text{HPO}_4^-]^4}{[\text{Ca}^{++}] [\text{H}_2\text{PO}_4^-]^2} = \frac{[\text{Ca}^{++}]^3 [\text{HPO}_4^-]^4}{[\text{H}_2\text{PO}_4^-]^2} = K_2 \quad (7)$$

Solving for Ca⁺⁺, we obtain,

$$\text{Ca}^{++} = K_2^{1/3} \cdot \frac{[\text{H}_2\text{PO}_4^-]^2}{[\text{HPO}_4^-]^4} \quad (8)$$

Considering the H_2PO_4^- as originating from any soluble, highly dissociable, monovalent, primary phosphate and assuming the average degree of ionization of the salt, BH_2PO_4 , to be γ_1 , and the HPO_4^- as originating from any soluble, highly dissociable, monovalent, secondary phosphate, B_2HPO_4 , with an average degree of ionization γ_2 ,

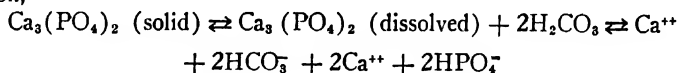
$$\text{Ca}^{++} = \left(K_2^{\frac{1}{2}} \cdot \frac{(\gamma_1)^{\frac{1}{2}}}{(\gamma_2)^{\frac{1}{2}}} \right) \cdot \frac{[\text{BH}_2\text{PO}_4]^{\frac{1}{2}}}{[\text{B}_2\text{HPO}_4]^{\frac{1}{2}}} = K'' \cdot \frac{[\text{BH}_2\text{PO}_4]^{\frac{1}{2}}}{[\text{B}_2\text{HPO}_4]^{\frac{1}{2}}} \quad (9)$$

Inverting and expressing in logarithmic form,

$$\text{pCa} = \text{pK}'' + \frac{2}{3} \log \frac{[\text{B}_2\text{HPO}_4]^{\frac{1}{2}}}{[\text{BH}_2\text{PO}_4]^{\frac{1}{2}}} \quad (10)$$

The value of pK'' and experimental data on the phosphates as calcion buffers will be reported in a separate paper.

In the presence of both carbonates and phosphates, the calcion concentration can be derived from either independently. Consider the reaction,



The law of mass action gives,

$$\frac{[\text{Ca}^{++}]^3 [\text{HCO}_3^-]^2 [\text{HPO}_4^-]^2}{[\text{H}_2\text{CO}_3]^2} = K_3 \quad (11)$$

since the concentration of dissolved $\text{Ca}_3(\text{PO}_4)_2$ in equilibrium with the solid phase is constant. From the first dissociation equilibrium of carbonic acid, we have,

$$\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = \frac{K_4}{[\text{H}^+]}$$

Substituting in Equation 11,

$$\frac{[\text{Ca}^{++}]^3 [\text{HPO}_4^-]^2}{[\text{H}^+]^2} = \frac{K_3}{(K_4)^2} = K_5$$

Solving for Ca^{++} ,

$$\text{Ca}^{++} = K_5^{\frac{1}{3}} \cdot \frac{[\text{H}^+]^{\frac{2}{3}}}{[\text{HPO}_4^-]^{\frac{2}{3}}}$$

From the second dissociation equilibrium of phosphoric acid, we have,

$$[\text{H}^+] = K_6 \frac{[\text{H}_2\text{PO}_4^-]}{[\text{HPO}_4^-]}$$

Substituting this equivalent of $[\text{H}^+]$ in the above equation,

$$\text{Ca}^{++} = K_7 \frac{[\text{H}_2\text{PO}_4^-]^3}{[\text{HPO}_4^{--}]^3}$$

which is identical with Equation 8.

The General Equation for the Calcion Concentration of Calcion Buffering Solutions.—Equations 3 and 4, expressing the buffering reactions of the carbonates and phosphates, are special forms of the general equation for any buffering solution,

$$a\text{Ca}^{++} + 4(\text{H}_{a-(x+1)}\text{A}) = \text{Ca}_a\text{A}_2 + 2(\text{H}_{a-x}\text{A})$$

where

x = an arbitrary valence number $< a$.

a = basicity of the acid; $a = x + 2$.

H_{a-x}A = free buffer acid.

$\text{H}_{a-(x+1)}\text{A}$ = acid ion of the primary acid.

Ca_aA_2 = insoluble normal calcium salt of the buffer acid.

The law of mass action applied to the above equation gives,

$$\frac{[\text{Ca}^{++}]^a [\text{H}_{a-(x+1)}\text{A}]^4}{[\text{H}_{a-x}\text{A}]^2} = K$$

since the concentration of the dissolved Ca_aA_2 in equilibrium with the solid phase is constant. Solving for Ca^{++} and expressing the ionic forms whose valences are u and $u + 1$ respectively, in terms of their soluble, highly dissociable, monobasic salts,

$$\text{Ca}^{++} = K. \frac{[\text{B}_u\text{H}_{a-x}\text{A}]_a^{\frac{2}{a}}}{[\text{B}_{u+1}\text{H}_{a-(x+1)}\text{A}]_a^{\frac{4}{a}}} \quad (12)$$

Inverting and expressing in logarithmic form,

$$\text{pCa} = \text{pK} + \frac{2}{a} \log \frac{[\text{B}_{u+1}\text{H}_{a-(x+1)}\text{A}]^2}{[\text{B}_u\text{H}_{a-x}\text{A}]} \quad (13)$$

The coefficient, $\frac{2}{a}$, is the ratio of the valence of calcion to the valence of acid, a . When the buffer acid, $[\text{B}_u\text{H}_{a-x}\text{A}]$, is free acid then u becomes zero, B_u disappears and the buffer acid is represented simply by H_{a-x}A . Equations 12 and 13 may be expressed more conveniently if we represent the concentration of free buffer acid by HA , the concentration of buffer salt by BA , and the valence ratio $\frac{2}{a}$, by n .

$$\text{Ca}^{++} = K \frac{[\text{HA}]^n}{[\text{BA}]^{2n}} \quad (14)$$

and

$$\text{pCa} = \text{pK} + n \log \frac{[\text{BA}]^2}{[\text{HA}]} \quad (15)$$

The calcion concentration is, therefore, dependent upon the ratio of the square of the salt concentration to the acid concentration. Equations 5, 6, 8, and 10 for the carbonates and phosphates are clearly special cases of the general equations Nos. 14 and 15, expressing the calcion concentration in terms of any buffering polyvalent acid and its soluble salt.

The Unit for Evaluation of Calcion Buffers

The measure of the stability of the calcion concentration or the calcion buffer value can be derived from the relations which govern the calcion concentrations in terms of its buffers. A consideration of the calcion buffering Equation 1 shows that equivalent increments of CaCl_2 produce equivalent decrements in buffer salt, BHA. Therefore, the unit for the calcion buffer value is the differential ration $\frac{d[\text{BA}]}{dp\text{Ca}}$ which gives the relationship between the increment of calcium salt added to a calcion buffer solution and the resultant increment in $p\text{Ca}$. This unit for calcion buffer value in terms of logarithmic $p\text{Ca}$ is analogous to that introduced by Van Slyke (2) for the hydrion buffer value and has similar advantages. The buffer value is the number of gram equivalents of buffer salt necessary to change the calcion concentration one unit of $p\text{Ca}$.

The General Equation for Calcion Buffer Value

The calcion buffer value may be determined by rewriting Equation 14,

$$\text{Ca}^{++} = K \frac{([\text{C}] - [\text{BA}])^n}{[\text{BA}]^{2n}}$$

where $[\text{C}]$ is the total acid concentration and $[\text{C}] - [\text{BA}]$ is the free buffer acid concentration. Expressing in logarithmic form,

$$p\text{Ca} = pK - n \log \frac{([\text{C}] - [\text{BA}])}{[\text{BA}]^2}$$

Differentiating with respect to $[\text{BA}]$ and inverting, we obtain for the calcion buffer value, ρ ,

$$\rho = \frac{d[\text{BA}]}{dp\text{Ca}} = -\frac{1}{n} \cdot \frac{d[\text{BA}]}{d \log \left(\frac{[\text{C}] - [\text{BA}]}{[\text{BA}]^2} \right)} = \quad (16)$$

$$\frac{2.3}{n} \cdot \frac{[\text{BA}] ([\text{C}] - [\text{BA}])}{2 [\text{C}] - [\text{BA}]}$$

From the above and from the Henderson-Hasselbalch equation,

$$[BA] = \frac{K'a}{K'a + [H^+]} [C]$$

and

$$[HA] = \frac{[H^+]}{[H^+] + K'a} [C]$$

where $K'a$ is the acid dissociation constant divided by the fraction of the salt ionized. For polybasic acids, the concentration of the acid and basic intermediate compounds may be expressed by similar but more complex ratios. Within physiological conditions, however, the simpler relations suffice. For such systems, the $K'a$ value is the intermediate dissociation constant of the weak buffer acid divided by the fraction of the salt ionized.

Substituting these values in Equation 16 we obtain,

$$\left. \begin{array}{l} \text{Equivalent ex-} \\ \text{pressions for} \\ \text{the calcion buf-} \\ \text{fer value,} \\ \frac{d(BA)}{dpCa}, \text{ or } \rho. \end{array} \right\} \begin{array}{l} \frac{d(BA)}{dpCa} = \frac{2.3}{n} \cdot \frac{[BA] [HA]}{[C] + [HA]} \quad (17) \\ = \frac{2.3}{n} \cdot \frac{[BA] [HA]}{[BA] + 2[HA]} \quad (18) \\ = \frac{2.3}{n} \cdot \frac{K'a[H^+] \cdot [C]}{(K'a + [H^+])(K'a + 2[H^+])} \quad (19) \\ = \frac{2.3}{n} \cdot \frac{K'a[HA] [C]}{(K'a + [H^+])([C] + [HA])} \quad (20) \\ = \frac{2.3}{n} \cdot \frac{[H^+] [BA]}{(K'a + 2[H^+])} \quad (21) \end{array}$$

The accuracy of Equations 16 to 21 has been tested against experimental results by applying data reported in a previous paper (1). The values given in Table IX show satisfactory agreement between the theoretically calculated calcion buffer values, i.e. $\frac{d[BHCO_3]}{dpCa}$ or ρ , and those approximated from the experimental data for $\Delta BHCO_3$ and ΔpCa .

Deductions from the General Equations

These equations permit the following deductions:

(1) The calcion buffer value at a given hydron concentration is directly proportional to the total molal acid concentration, $[C]$ (Equation 19).

(2) The calcion buffer value at a given hydron concentration is directly proportional to the buffer salt concentration, $[BA]$ (Equation 21).

TABLE IX

VALUES OF $\frac{\Delta \text{BHCO}_3}{\Delta \text{pCa}}$ AS ESTIMATED FROM VALUES OF ΔBHCO_3 AND ΔpCa TAKEN FROM DATA (1), COMPARED WITH VALUES OF $\frac{\Delta \text{BHCO}_3}{\Delta \text{pCa}}$ CALCULATED BY EQUATIONS 16 TO 21

pCa	ΔpC	$[\text{BHCO}_3]$	Mean $[\text{BHCO}_3]$	$[\text{H}_2\text{CO}_3]$	Mean $[\text{H}_2\text{CO}_3]$	$[\text{C}]$	Mean $[\text{C}]$	$[\text{H}^+] \cdot 10^7$	Mean $[\text{H}^+] \cdot 10^7$	ΔBHCO_3	$\frac{\Delta \text{BHCO}_3}{\Delta \text{pCa}}$	$\frac{\Delta \text{BHCO}_3}{\Delta \text{pCa}}$ Calculated from Equations
2.82	0.18	0.0109	0.0105	0.00208	0.00172	0.01298	0.01206	0.99	0.80	0.0011	0.005	16-18
3.00	0.24	0.0098	0.0131	0.00136	0.00207	0.01114	0.01525	0.60	0.67	0.0066	0.003	19-21
3.24	0.06	0.0164	0.0163	0.00278	0.00243	0.01936	0.01882	0.73	0.48	0.0002	0.003	0.004
3.30	0.22	0.0162	0.0160	0.00208	0.00172	0.01828	0.01767	0.23	0.27	0.0005	0.002	0.003
3.52	0.20	0.0157	0.0156	0.00136	0.00102	0.01706	0.01661	0.31	0.24	0.0002	0.001	0.002
3.72		0.0155		0.00068		0.01616		0.17		0.0002		0.001

(3) The molal calcion buffer value, ρ_M , follows from Equation 19

$$\rho_M = \frac{d[BA]}{[C]dpCa} = \frac{2.3}{n} \cdot \frac{K'_a[H^+]}{(K'_a + [H^+])(K'_a + 2[H^+])} \quad (22)$$

The relationship between the absolute calcion buffer value, ρ , and the molal buffer value ρ_M , is

$$\frac{\rho}{[C]} = \rho_M$$

(4) The calcion buffer effect is independent of the nature of the buffer acid since Equations 16, 17, and 18 are independent of the dissociation constant of the acid. Phosphoric and carbonic acids have, in the presence of their salts, under similar conditions and equivalent concentrations corrected for valence differences, the same buffer effectiveness but their maximum calcion buffer action is exerted at different pH.

(5) *The Calcion Buffer Values of the Carbonates and Phosphates.*—These are given by Equations 16 to 21 in which the value of n is unity for the carbonates and two-thirds for the phosphates.

The Calcion Buffer Value of a Mixture of Calcion Buffers.—In a solution of a given hydrion concentration $[H^+]$, containing several calcion buffers, the following relations hold at equilibrium,

$$[H^+][A^+]_i = K_i([C]_i - [A^+]_i)$$

where $i = 1, 2, 3, \dots, n$

The condition of electroneutrality requires that,

$$[B^+] + [H^+] = [OH^-] + [A^-]_1 + [A^-]_2 + \dots + [A^-]_n$$

where $[B^+]$ represents the total equivalent concentration of buffer salt cations present in the solution; in this summation both $[H^+]$ and $[OH^-]$ may be neglected, as they are small in comparison with the other quantities. Substituting for the anion concentrations their equivalents,

$$[BA] = \left(\frac{K'_1[C]}{K'_1 + [H^+]} + \frac{K'_2[C]}{K'_2 + [H^+]} + \dots + \frac{K'_n[C]}{K'_n + [H^+]} \right)$$

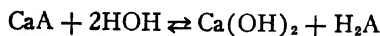
where the K' value is the intermediate dissociation constant divided by the fraction of salt ionized. Hence, the total buffer salt concentration is the sum of the separate buffer salts. Moreover, the differential coefficients of this sum of functions is equal to the sum of the differential coefficients of these functions, that is, the calcion buffer values,

$\frac{d(BA)}{dpCa}$, are additive.

A solution containing several calcion buffers has for its resultant calcion buffer value at a given hydrion concentration, the sum of the individual buffer values,

$$\rho = (2.3) [H^+] \left\{ \frac{1}{n_1 (K'a_1 + 2[H^+])} + \frac{1}{n_2 (K'a_2 + 2[H^+])} + \dots \right\} \quad (23)$$

The Calcion Buffer Value of an Aqueous Highly Dissociated Calcium Salt.—Addition of a highly dissociated calcium salt to water yields a solution which exerts a certain amount of calcion buffer value. This may be determined by considering that addition of a calcium salt to water results in an hydrolysis, according to the equation,



Here a molecule of CaA is transformed to a molecule of the calcium buffer salt, Ca(OH)₂. Calcium hydroxide is a relatively insoluble compound whose solubility product is of an order of 10⁻¹⁶

$$[Ca^{++}][OH^-]^2 = k_1$$

or

$$Ca^{++} = \frac{k_1}{[OH^-]^2}$$

Inverting and expressing in logarithmic form,

$$pCa = pK_1 + 2 \log [OH^-]$$

Differentiating,

$$dpCa = 2d \log [OH^-]$$

Since a molecule of CaA is equivalent to a molecule of Ca(OH)₂, we may express the addition of a calcium salt by the formation of the calcium buffer salt, Ca(OH)₂, or symbolically by 2 BA
But,

$$BA \approx \frac{1}{2} Ca(OH)_2 \approx \frac{1}{2} \frac{2[OH^-]}{\gamma_b}$$

where γ_b represents the degree of dissociation of Ca(OH)₂.
Differentiating,

$$d[BA] = \frac{d[OH^-]}{\gamma_b}$$

Therefore,

$$\rho = \frac{d[BA]}{dpCa} = \frac{1}{\gamma_b} \frac{d[OH^-]}{dpCa} = \frac{1}{\gamma_b} \cdot \frac{d[OH^-]}{2d \log [OH^-]} = \frac{1}{2\gamma_b} \cdot \frac{[OH^-]}{0.4343} = \frac{1.15}{\gamma_b} [OH^-] \quad (24)$$

It can be similarly derived that,

$$\frac{d[BA]}{dpCa} = \frac{1.15}{\gamma_a} [H^+] \quad (25)$$

where γ_a is the degree of dissociation of the acid. Adding Equations 24 and 25, respectively, we obtain the total calcion buffer value of an aqueous, highly dissociated calcium salt solution.

$$\rho_w = \frac{d[BA]}{dpCa} = 1.15 \left(\frac{[H^+]}{\gamma_a} + \frac{[OH^-]}{\gamma_b} \right) \quad (26)$$

This means that any given $[H^+]$ or $[OH^-]$, highly dissociated calcium salt must be added at the rate of 1.15 gram equivalents per liter per unit change in PCa effected.

The total calcion buffer value of a weakly dissociated calcium salt solution to which highly dissociated calcium salts are added is the sum of the calcion buffer values of the weakly and the strongly dissociated calcium salts. This follows from Equation 23 and is given by the sum of Equations 22 and 25,

$$\rho = 1.15 \left(\frac{2K'_a[C][H^+]}{(K'_a + [H^+])(K'_a + 2[H^+])} + \frac{[H^+]}{\gamma_a} + \frac{[OH^-]}{\gamma_b} \right) \quad (27)$$

Since $[H^+]$ and $[OH^-]$ are usually negligible in comparison with the first member of Equation 26, the use of ρ_M becomes permissible within the physiological range.

The Maximum Calcion Buffer Values

The maximum calcion buffer value is determined when $\frac{d\rho}{d[BA]}$ becomes zero. Repeating the differentiation of Equation 16 we obtain,

$$\frac{d\rho}{d[BA]} = \frac{1}{\log_{10}e} \left(1 - \frac{2[C]^2}{(2[C] - [BA])^2} \right) \quad (28)$$

When this expression becomes equal to zero

$$[BA] = (2 \pm \sqrt{2})[C] \quad (29)$$

To determine which of these two values represents the maximum the necessary and sufficient condition is that $\frac{d^2\rho}{d[BA]^2} < 0$ for that value. Repeating the differentiation of Equation 28,

$$\frac{d^2\rho}{d[BA]^2} = \frac{-4[C]^2}{(2[C] - [BA])^3} \quad (30)$$

Substituting $(2 - \sqrt{2}) [C]$ from Equation 29 in Equation 30 a negative value is obtained; for $[C]$ is always positive. Hence the maximum calcion buffer value is determined when

$$[BA] = (2 - \sqrt{2})[C] = 0.586 [C] \quad (31)$$

Hence the maximum calcion buffer effect is produced by any calcion buffering, weak acid-salt solution containing 0.586 part of buffer salt to 0.414 part of free buffer acid.

The Molal Calcion Buffer Value at the Maximum.— ρ_M at the point of maximum calcion buffering may be calculated from Equations 17, 18, and 19 by substituting for $[BA]$ its equivalent $0.586 [C]$ from Equation 28. Hence,

$$\rho_M = \frac{2.3}{n} \cdot \frac{[BA] [HA]}{[C] + [HA]} = \frac{0.395}{n} \quad (32)$$

for any calcion buffering, weak acid-salt solution. Since the value of n for the carbonates is unity and for the phosphates is two-thirds, *the molal calcion buffer value for the carbonates is 0.395 and for the phosphates is 0.592*. At the maximum a molal solution of phosphates buffers the calcion concentration one and one-half times better than a molal solution of carbonates. The meaning of these values may be ascertained from the buffering reaction, from which it is evident that for every molecule of calcium salt added to the calcion buffering solution, a molecule of calcium buffer salt is formed which is transformed into a molecule of the insoluble normal calcium salt. Hence, at the isohydric point of maximum buffering, a highly dissociated calcium salt must be added or removed to the extent of 0.395 M to carbonates and 0.592 M to phosphates, to change the original calcium concentration to 10-fold or 1/10 its own value, and thereby cause one unit change in PCa.

The Relation of pH to Maximum Calcion Buffer Value.—The pH of a buffer solution at which its molal calcion buffer value is a maximum, may be determined directly from the Henderson-Hasselbalch equation,

$$\text{pH} = \text{pK}'a + \log \frac{[BA]}{[HA]}$$

Since $[BA]$ and $[HA]$ are 0.586 and 0.414, respectively, at the maximum, the equation becomes,

$$\text{pH} = \text{pK}'a + \log \sqrt{2} \quad (33)$$

This equation may also be derived from the equivalent expressions given by Equations 19 to 21, by substituting the calculated ρ_M values at the maximum from Equation 32; *e.g.*,

$$\frac{2.3}{n} \cdot \frac{[BA] [H^+]}{(K'a + 2[H^+])} = \frac{2.3}{n} \frac{(0.586) [H^+]}{(K'a + 2[H^+])} = \frac{0.395}{n}$$

Solving for H^+ , we obtain,

$$H^+ = 0.7 K'a$$

Inverting and expressing in logarithmic form,

$$pH = pK'a + \log \sqrt{2}$$

which is identical with Equation 33.

The independent derivation of this equation follows from direct differentiation of ρ given by Equation 22,

$$\frac{d\rho_M}{dCa} = \left(\frac{d\rho_M}{d[H^+]} \right) \left(\frac{d[H^+]}{dII} \right) \left(\frac{dH}{dCa} \right) \quad (34)$$

Differentiating ρ_M with respect to H ,

$$\frac{d\rho_M}{d[H^+]} = \frac{2.3 (K'a^3 - 2 K'a[II^+])^2}{(K'a + [H^+]) (K'a + 2[H^+])^2}$$

Since,

$$\frac{d[H^+]}{dH} = -2.3 [H^+]$$

and from the relations,

$$Ca^{++} = \frac{k}{[OII^+]} = \frac{k_1}{k_2^2} [H^+]^2 = k_2 \cdot [II^+]^2$$

or

$$pCa = pK_2 - 2 \log [H^+]$$

and on differentiating,

$$\frac{d[H^+]}{dCa} = -\frac{1}{2}$$

Equation 34 becomes on multiplying these three differentials,

$$\frac{d\rho_M}{dCa} = \frac{1.15 [H^+] (K'a^3 - 2 K'a[H^+])^2}{(K'a + [H^+]) (K'a + 2[H^+])^2}$$

Making this equation equal to zero,

$$H^+ = \pm \frac{K'a}{\sqrt{2}}$$

Proceeding as above to determine which of these values is a maximum, inverting and expressing in logarithmic form, we obtain,

$$pH = pK'a + \log \sqrt{2}$$

The ratio of buffer salt to free buffer acid is the same for any calcion buffering solution at the maximum and hence the pH at that point for any buffer concentration may be calculated from Equation 33 by using $pK'a$ values of 6.15 for the carbonates and 6.85 for the phosphates. *The maximum calcion buffer value for the carbonates is at pH 6.30 and that for the phosphates is at pH 7.00.*

The Molal Calcion Buffer Value at the pH of the Blood.—The carbonates and phosphates exert their maximum calcion buffer effect at an hydriion concentration greater than that of normal blood. The calcion buffer value at pH 7.35 may be calculated from Equation 19. At pH 7.35 the molal calcion buffer value of the carbonates is 0.111 or 28 per cent of the maximum. The molal calcion buffer value of the phosphates is 0.265 or 45 per cent of the maximum.

The Calcion Buffer Value of Blood Serum

The calcion buffer value of blood may be calculated from Equations 17 to 22. Since the corpuscles contain no calcium, calcion buffering is limited to the serum. Normal blood serum has a total bicarbonate concentration of about 0.03 N and an hydriion concentration of 0.45×10^{-7} . Hence, the calcion buffer value of the serum carbonates is 3.5×10^{-8} from

$$p = \frac{d[BA]}{dpCa} = \frac{2.3}{n} \frac{K'a[H^+] \cdot [C]}{(K'a + [H^+])(K'a + 2[H^+])}$$

At the same hydriion concentration and at a total phosphate concentration of 0.001 M the calcion buffer value of the serum phosphates is 0.5×10^{-8} . The combined calcion buffer value of the carbonates and phosphates of normal blood serum according to Equation 23 is 4.0×10^{-8} . Therefore, at physiological conditions, the calcion buffer value of the carbonates is seven times that of the phosphates.

DETERMINATION OF IONIC CONCENTRATION BEFORE AND AFTER CLOTTING

Conductivity measurements showed a distinct decrease in conductivity after clotting and even after hot-washing the clot from solution held loosely by it. It was planned to develop alkali-metal amalgam electrodes to enable the exact determination of ion concentration by the potentiometer chain method but in the meantime the next best procedure was to apply the solubility-product principle to the ion concentration in solution before and after clotting using the ultrafiltrate in each case.

From the solubility- or ion-product principle we know that in gen-

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eral for a saturated solution of a difficultly soluble salt at a given temperature the product of the ion concentration, each raised to the power corresponding to the number of that kind of ion formed from the ionization of one molecule of the salt, is a constant. Applying this method it is only necessary to determine the concentration of the added ion to the point of turbidity, *i.e.*, the concentration at which the solubility product of the compound is just exceeded. It is of no final consequence if other ions present can precipitate with the added ion. The necessary condition however is that the concentration of the ion sought from the most insoluble substance which can result in the solution. If the solubility product of the precipitate formed be known it is a matter of simple arithmetic to calculate the concentration of its ion in solution.

The components necessary and sufficient for clotting were prepared as usual and divided into two parts. One portion was ultrafiltered by inverse suction method.²¹ The ultrafilter consisted of a Gooch crucible to the bottom of which was sealed by means of stick shellac a specially prepared membrane. The membrane mixture was made from a modified pyroxylin solution as follows:

3% collodion 10 parts
Ethyl acetate 1 part
Glycerine 0.5 part

Addition of ethyl acetate yields a clearer, higher water content, and therefore more easily wettable, *i.e.*, increased permeability. Glycerine prevents the decrease in permeability by the air.

Calcium ion content was determined by bringing the solution to a hydron concentration of 0.3×10^{-7} on adding N/100 NaHCO₃. The N/100 sodium oxalate was added from a burette unto the first indication of permanent turbidity. The chlorion concentration was determined by adding in the same way N/100 silver nitrate solution. The results obtained are given in Table X below.

TABLE X
IONIC CONCENTRATION IN MGMS. PER TEN CC. OF SOLUTION BEFORE AND AFTER CLOTTING

Calcion Conc.		Chlorion Conc.	
Before	After	Before	After
0.74	0.31	31.5	26.0

Solubility Products

Ca₂O₄— 1.75×10^{-8}

AgCl— 1.56×10^{-10}

An analysis of the results above with due consideration to the limits of experimental error, points clearly to two facts—a portion of the

²¹ I. Newton Kugelmass, Johns Hopkins University Dissertation, 1921.

calcium concentration is bound during clotting whereas the chlorine concentration remains practically unaltered.

VI. CHANGES IN THE PROTECTIVE POWER OF THE PROTEIN CLOTTING SYSTEM

Emulsoid colloids when added in comparatively minute quantities to suspensoids have the power of preventing the coagulation of the suspensoid particles. Surface tension relations between the medium, the suspensoid particles and the emulsoid substance, result in the latter surrounding the suspended particle with a thin coating which prevents the coalescence of the particles either by preventing the discharge of the particles or merely by offering a material obstacle to coalescence.

The degree of protective action of emulsoids for colloidal gold is expressed by what Zsigmondy has termed the Gold Number. It is the weight in mgms. of a colloid which just fails to prevent the change from red to violet in ten c.c. of a gold solution when one c.c. of a ten per cent solution of NaCl is added to it.

To follow the change in the protective effect of the proteins for colloidal gold a sufficient quantity of thrombin and plasma solutions were prepared and a series of tubes of approximately equal bore arranged. A solution of one cc. of thrombin added to two cc. of plasma was prepared for each five-minute time interval study. Quantities of solution were added in the order given in the table below with a few minutes' time interval before the addition of aq NaCl. The concentration of clotting solution which brought about the color transition in the gold hydrosol from red to violet was taken as the "gold number" of that sample of clotting system. The same procedure was carried out with clotting solutions pipetted out after definite time intervals. (Table XI.)

TABLE XI

Components of Mixture	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
Gold hydrosol . . .	1.00	1.00	1.00	1.00	1.00
Distilled water	0.10	0.30	0.40	0.45	0.48
Clotting solution	0.40	0.20	0.10	0.05	0.02
10 per cent. NaCl	1.00	1.00	1.00	1.00	1.00

The result of this study showed clearly the protective power of the emulsoids in the clotting system increased as clotting proceeded and a maximum after syneresis of the clot from the protein system has taken place. Definite quantitative data however were difficult to obtain. The method of procedure was then modified, keeping constant the

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quantities of gold hydrosol and clotting solution, but varying the aq NaCl solution and moreover color change was detected in a colorimeter.

A standard solution whose composition was analogous to that of the clotting system was made up of 5 cc. of gold hydrosol to which was added 0.1 cc. of a solution composing (E.P.Ca. (1) : E.P.(1.5)) preparation was just at the color transition of the gold hydrosol from red to violet and served as the standard in one tube of the colorimeter. In the other colorimeter tube was poured 5 c.c. of the gold hydrosol, a tenth of a cc. of cbt. solution mixed and then gradually a ten per cent

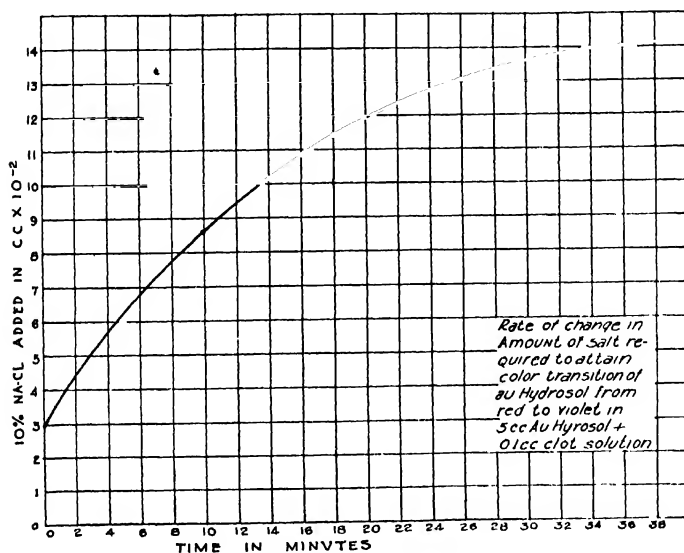


FIG. 4.

sodium chloride solution was added until the colors matched. The quantities of salt added are clearly functions of the protective power of the emulsoid system. (Table XII, Fig. 4.)

Increase in the protective power of the proteins as coagulation proceeds, attaining a maximum after syneresis, is again substantiated with the added satisfaction of having specific quantitative data. This behavior runs parallel with the already established results of diminution in conductivity and diminution in hydron concentration. Increase of protective power of hydrophile substances in general means:

- (a) An increase in the degree of the dispersion of the protein.

TABLE XII

AMOUNTS OF 10 PER CENT NaCl NECESSARY TO ATTAIN COLOR TRANSITION OF GOLD HYDROSOL FROM RED TO VIOLET IN 5 CC. GOLD SOL—0.1 CC. CLOT SOLUTION

Time in Minutes	10 Per Cent NaCl Added in cc. $\times 10^{-3}$
0	3.0
10	8.5
18	11.2
26	13.0
36	14.0
Exuded liquid from washed clot.....	11.0

- (b) A decrease in the ionic concentration of the protein.
- (c) An increase in the viscosity of the protein.
- (d) An increase in stability of the protein.
- (e) An increase in the adsorbability of protein.
- (f) An increase in their power to lower the surface tension of the dispersion medium.
- (g) An increase in the hydrophylic state of the protein.

VII. INFLUENCE OF CONCENTRATION ON RATE OF CLOTTING

Optimum clotting is obtained, keeping all other conditions constant, on mixing solutions of thrombin and plasma as prepared above in the ratio of 2:1 by volume. There must be then in the thrombin solution one or more components whose optimum concentrations control the rate of clotting. It was the present purpose then to establish the relationship, if existent, between thrombin concentration and plasma and further, the concentration of the necessary and sufficient thrombin components for clotting.

Clotting time determinations were made at 39° C. on a series of mixtures containing the same amount of plasma and varying concentrations of the thrombin solution as recorded.

TABLE XIII

EFFECT OF THROMBIN CONCENTRATION ON CLOTTING TIME

Mixture Examined	Exp. 1	Exp. 2 Clotting	Average Time	Rate of Reaction Constant
2.0 cc. th. + 1 cc. pl....	44	36	40	1.35 1.28 1.30
1.0 cc. P.S. + 1.0 " " + 1 " "....	50	50	50	
1.5 " P.S. + 0.5 " " + 1 " "....	76	73	75	
1.75 " P.S. + 0.25 " " + 1 " "....	110	—	110	
1.88 " P.S. + 0.12 " " + 1 " "....	—	—	—	

P.S. = Physiologic saline; th. = solution of thrombin; pl. = plasma.

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The values representing the influence of concentration of thrombin on plasma cannot be reduced to the ordinary equations of homogeneous or heterogeneous reactions. The exponential expression $c^kt = \text{constant}$ where c = the thrombin concentration, t , the clotting time and k , a constant. The greater k is the more rapidly the velocity of reaction increases with concentration. On actual application of the equation to the clotting system, we have

$$c_0^k t_0 = c_1^k t_1 \text{ or } \frac{t_0}{t_1} = \left(\frac{c_1}{c_0} \right)^k \text{ or } k = \frac{\log \left(\frac{t_0}{t_1} \right)}{\log \left(\frac{c_1}{c_0} \right)}$$

Very satisfactory agreement was obtained by this equation as shown by the negligible deviation of k (*vide* Table XIII).

Instructive as the mathematical expression may be generally its physical significance as far as the clotting system is concerned cannot be derived therefrom in sufficiency to throw light on its mechanism, in view of the empiracy of the equation. It is this. What concentration of components in thrombin solution must be maintained constant in order to reduce this equation to its monomolecular form $ct = \text{constant}$, *i.e.*, where the exponential constant $k = \text{unity}$. Some preliminary experiments have shown that varying the concentration of thrombin but maintaining the calcion concentration identical in each system, gave the desired results.

Based on this principle the solutions were prepared thus: 0.5 cc. cytozyme, 1.5 serum issued from very limpid plasma, and 5 cc. P. S. are mixed. This solution of thrombin differs from the ordinary thrombin solution in that it is free from Ca^{++} . This solution is divided in two parts. In five tubes are added respectively 2.8; 1.4; 0.7; 0.35 and 0.17 cc. of this solution and to each is then added P.S.Ca conc. (1.15 gm. CaCl_2 per liter P. S.) and finally P. S. making the volume in each 4 cc. Each of these mixtures is maintained at 20°C . in a glass thermostat. They thus have concentrations of thrombin varying in the proportions: 2; 1; 0.5; 0.25; 0.12 while the Ca ion concentration is constant. To each of these tubes is now added 2 cc. oxalated plasma.

TABLE XIV

Components of the Mixture	Soln. 1 Conc. Thrombin — 2.0	Soln. 2 Conc. Thrombin — 1.0	Soln. 3 Conc. Thrombin — 0.5	Soln. 4 Conc. Thrombin — 0.25	Soln. 5 Conc. Thrombin — 0.12
Soln. thrombin	2.8 cc.	1.4 cc.	0.7 cc.	0.35 cc.	0.18 cc.
P. S. Ca conc.....	0.07 "	0.07 "	0.07 "	0.07 "	0.07 "
Physiol. saline	1.13 "	2.53 "	3.23 "	3.58 "	3.75 "
Oxalated plasma	2.0 "	2.0 "	2.0 "	2.0 "	2.0 "

The five mixtures in the tubes are now placed in a glass thermostat at 38° C. and the clotting times recorded. The table below gives the results representative of two of such a series.

TABLE XV
EFFECTS OF THROMBIN CONCENTRATION ON THE COAGULATION TIME MAINTAINING
THE CALCIUM ION CONCENTRATION CONSTANT

Thrombin Concentration	Clotting Time		Average	Ct. = Constant
	Expt. 1	Expt. 2		
2.00	34	26	30	60
1.00	67	53	60	60
0.50	116	115	115	57
0.25	—	250	250	62
0.12	—	—	—	—

Thus diluting the thrombin solution there is a decrease in the concentration of the three components: Serum, calcium ions, and cytozyme. The mathematical expression representative of the rate of clotting as a function of the component concentration has been demonstrated above (Table XV) to be general relation ct^k — constant.

Maintaining the Ca ion concentration constant in a series of diminishing thrombin solution concentrations reduces the exponential form of this expression to ct — constant. Using a very potent solution of cytozyme (Sumner) where one part in ten million is sufficient for coagulation, the non-exponential expression still holds if both calcium and cytozyme are maintained constant. These experiments demonstrate beyond doubt that a single component of the thrombin solution determines the time course of the clotting reaction. This component is associated with the serum albumin and globulin and is related to them in its physical properties. It is the prothrombin of Howell, the serozyme of Bordet, the thrombogen of Schmidt or Morawitz.

The kinetic behavior of the clotting system is so analogous in the essentials to the heterogeneous catalysis studies of Langmuir that an analysis of our results from such a standpoint leads to the following considerations:

1. The surface of the colloidal substance associated with serum albumin and globulin (serozyme of Bordet or prothrombin of Howell) which determines the time course of the clotting reaction has properties analogous to those of the surface of a solid.

2. The fibrinogen particles are adsorbed on the surface of this determining substance which is dispersed and contains nuclei for condensation.

3. A molecule of the adsorbed fibrinogen occupies an elementary

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unit space about the serozyme forming a system of spherical aggregates.

4. Jelling becomes evident when the total available surface of serozyme for condensation of the fibrinogen particles is completely covered and these spherical aggregates touch one another.

5. Continuous reticulum formation is ultramicroscopically evident when these aggregates coalesce into a single system forming chains.

VIII. CHANGES IN VISCOSITY IN THE COURSE OF COAGULATION

Paralleling our results with those of Langmuir on heterogeneous equilibria gives us a tentatively rather gross but clear picture of the mechanism of the clotting course. To confirm the types of particles aggregating in the clotting system, the changes in the viscosity of such a system in the course of coagulation were followed. Although we know very little of the theory of viscosity changes produced in a medium by dissolving substances which produce even true solutions still, as pointed out by Freundlich²² as long as we use viscosity for comparative experiments alone it is of the greatest value. We can detect easily very slight changes in solutions by viscosity measurements.

The procedure for measuring viscosity was twofold. The changes in viscosity from the time of mixing to the time of jelling were determined by Scarpa's²³ viscosimeter at constant temperature and constant negative pressure. The changes in viscosity during clotting were measured by means of a specially devised torsion viscosimeter²⁴ based on the principle of torsion of concentric cylinders. The results obtained for the course of clotting in a single series of experiments were calculated combining the values obtained from the two apparatuses by initial calibration of the results of one in terms of the other.

In a first series of experiments a thrombin and plasma solution were mixed in the usual way in a viscosity tube at constant temperature and the viscosity changes followed with time. The change in viscosity during clotting was surprisingly small. The resultant product was not the usual meshed reticulum but consisted of separate fibrils. The comparative standard, however, allowed to clot without disturbance, gave an excellent clot. Explanation of the results obtained is clear. Any stirring, shearing or flow through a capillary tube involves the deformation of any molecular structure forming within the fluid and thereby shows little change in viscosity. It does not however become impossible to measure the viscosity changes as they actually proceed in the clotting system.

²² H. Freundlich and N. Ishizaka, Die Koagulationsgeschwindigkeit von $Al(OH)_3$ Solen gemessen an der Aenderung ihrer Zähigkeit, *Kolloid-Zeit.*, 12, 280-288 (1918).

²³ S. Bottazzi, "In der Harn," Carl Neuberg, Berlin, 1911, t. II, pp. 1622-1625.

²⁴ I. Newton Kugelmann, Un viscosimetre a torsion pour les sols lyophiles, *C. R. Soc. Biol.*, 87, 885-888 (1922).

TABLE XVI
CHANGES IN VISCOSITY DURING COAGULATION

Time in Minutes	Experiment I				Experiment II			
	Initial pH of the Coagulating System—6.8				Initial pH of the Coagulating System—5.5			
	Time Flow Upward	Time Flow Downward	Viscosity Arbitrary Units		Time Flow Upward	Time Flow Downward	Viscosity Arbitrary Units	
0	15.0	46.0	11.4		14.7	53.0	11.2	
15	15.2	46.2	11.8		15.2	58.2	—	12.0
21	—	—	—		—	—	—	—
30	15.5	54.3	12.2		—	—	—	—
40	16.0	69.3	13.0		16.0	51.4	12.2	
54	16.5	101.4	14.2		—	—	—	—
60	—	—	—		16.2	70.5	—	13.3
Clot After 55 Minutes								
	Scale Leading		Difference (n)	Time of Rotation		Viscosity		
	Initial	Final		(t)	(nt)		(t)	(nt)
60	40	100	60	12	780	17.0	—	—
63	40	120	80	12	960	21.0	—	—
70	40	131	91	12	1092	23.8	—	—
73	—	—	—	—	—	—	—	—
77	40	116.7	76.7	12	920	20.0	12	985
81	—	—	—	—	—	—	—	—
83	—	—	—	—	—	—	12	1140
94	—	—	—	—	—	—	12	1090
Exuded Liquid—"Serum"							Exuded Liquid—"Serum"	
10.8							10.0	

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The procedure then pursued was to measure the viscosities of separate samples of the same preparation after various time intervals. Since only two cc. of solution are necessary for a measurement it was a simple matter to obtain results.

The values recorded and the curves representing them are very instructive. The viscosity was found to increase very slowly at first, then at the point of clot indication very rapidly till a maximum was reached beyond which syneresis began, and the viscosity diminished again. The same experiment was carried out with the mixture at a

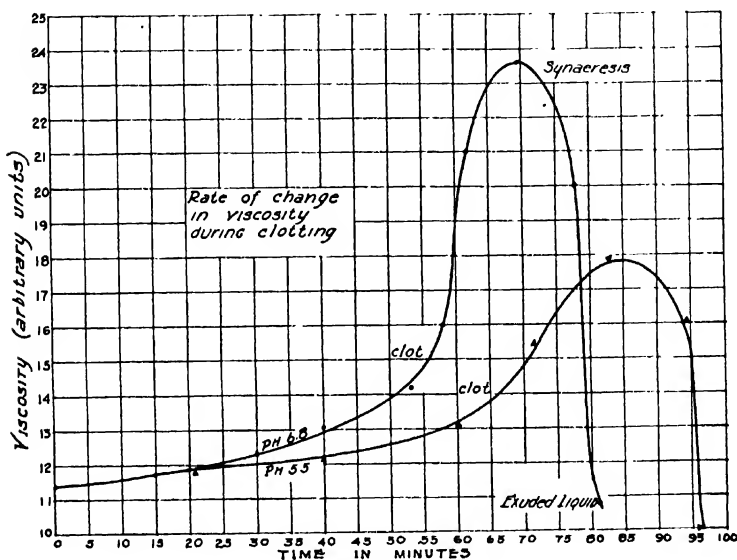


FIG. 5.

cH — 0.30×10^{-5} or a pH — 5.5. The viscosity curve is of the same general type but clot indications begin at a later time and the viscosity never reaches the magnitude that is attained at pH 6.8. Evidently an increase in the hydrion concentration or ionogen concentration in the solution lowers the viscosity and its maximum value and the product obtained is flaky rather than a continuous reticulum.

These changes in the course of clotting show that the process comprises two distinct stages: The latent period or that of precoagulation, relatively slow and the coagulating period relatively rapid.

General interpretation of these distinct stages in clotting results

from the works of Einstein,²⁵ Smoluchowski,²⁶ Arrhenius,²⁷ Freundlich.²⁸ The latent period detected by the relative slight increase in viscosity corresponds to the formation or growing of nuclear particles into spherical units of greater magnitude in which the dispersion medium filling the intermolecular or intraparticle spaces no longer behaves as free solution. The slow growth of these spherical units is reflected by the slow changes in viscosity which are a direct function of the size of these spherical particles. As these spherical units increase in both size and number mutual contact among them results. It takes more or less complete envelopment of these nuclei for the spherical units to realize contact amongst them. When this is attained to a great extent spontaneous jelling results. The process from this aggregate contact on consists in rapid readjustment of equilibria resulting in diffuse chain formations in various directions apparent ultramicroscopically and to the naked eye as reticulæ.

This is truly in accord with our expectations already cited in the previous concentration studies, *e.g.*, during the latent period or the stage of precoagulation, the serozyme particles acting as nuclei adsorb the fibrinogen particles until complete spherical units are formed. Once this is realized the clotting period sets in when rapid agglomerations of these spherical units take place evinced by the appearance of fibrillæ or reticulæ and confirmed by the enormous sudden increase in viscosity as a result of such continuous chain formations. In the first or latent period precoagulation is completely reversible for all molecular orientation takes place in a *hydrophile* medium. Once the total available surface of the serozyme particles becomes covered then the electronegation repulsion among the serozyme particles is thus decreased, a minimum thereby affecting the Brownian movement and stability of the system corresponding to the *hydrophobe transition* in coagulation. As a result, mutual contact takes place either by extension of the fibrinogen particles into the medium or by coalescence due to instability of serozyme nuclei. This contact of the particles through results in a continuous but porous aggregation, fibrillar in form, reticular in consistency. It is the rapid irreversible clotting period.

From the viscosity studies some further general conclusions may be derived as follows:

1. The course of clotting is autocatalytic.
2. Initial incubation period is influenced by thrombin concentration.
3. Existence of lower concentration limit of thrombin for clotting.

²⁵ A. Einstein, Eine neue Bestimmung der Molekül-dimensionen, *Ann. d. Physik.* (4), 19, 289-306 (1906).

²⁶ M. von Smoluchowski, Theoretische Bemerkungen über die Viskosität der Kolloide, *Kolloid Zeits.*, 18, 190-195 (1916).

²⁷ S. Arrhenius, The viscosity of solutions, *Biochem. Journ.*, 11, 112-118 (1917).

²⁸ A. Meyer, Beiträge zur Kenntniss der Gallerten besonders der Starke Gallerten, *Kolloidchemische Beihefte*, 5, 1-48 (1918).

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4. Incubation period is practically infinite below certain thrombin concentration.

5. Existence of critical concentration range for optimum clotting.

6. Optimum clotting system shows:

(a) Relatively long incubation period.

(b) Relatively short clotting period.

7. Inflection point of system (a) is elevated by homogeneous viscous liquids—sugar, glycerin; (b) is decreased by hydrions.

8. Particles separate by syneresis as structured reticulum.

9. Coagulum is an elastic clot. Elasticity is increased by sugar and glycerin, decreased by hydrions and salts.

Some of the factors responsible for the incubation period in the clotting system are:

1. Forces that decrease the degree of swelling of fibrinogen particles.

2. Growth of micron fibrin needles at the expense of sub- and amicrons respectively.

3. Orientation of needles longitudinally to form filaments and transversely to form films.

4. Degree of wetting of the solution toward a protuberant glass surface or the degree of non-wetting toward a smooth paraffin surface and the total surface wetted by the solution. The solution spreads over the wetted surface, is adsorbed by it and within this thin film reactivity and necessary orientation proceed at more rapid rate than within the solution. Once begun this active film becomes the nucleus from which reactivity, growth and orientation of the particles proceed fast in the direction of the solution.

IX. CHANGES IN TRANSPARENCY DURING COAGULATION

Direct relationships exist between the degree of dispersion of a colloidal system and certain of its intrinsic properties. Changes in these are strictly parallel to simultaneous changes in the degree of aggregation.

That such relationship exists is evident on analysis of the works of Freundlich²² and Ishizake on aluminum hydroxide sols, of Meyer²³ on starches, of Arisz²⁹ on gelatine, of Lottermosser³⁰ on tungstic acid. These studies point to the direct possibility of determining degree of aggregation of colloidal systems by measuring the index of transparency. Such optical measurements interpreted in terms of dispersion changes in blood clotting systems would add to the understanding of the interior

²² L. Arisz, Over het Tyndallverschynsel in gelatineplussingen., *K. Akad. v. Wetensch te Amsterdam. Vergl. vergaden Wissen. Natuurk. af.*

³⁰ A. Lottermosser, Researches on the precipitation, *Kolloid. Zeitsch.*, **4**, 140-148 (1914).

mechanism of the particles involved. The nephelometric methods are insensitive and are subject ¹⁸ to errors of personal equation and so a new apparatus was devised. Its assemblage consists of (a) standard intensity lamp whose rays are emitted parallel by means of a convex lens (b) parallel faced cuvette containing colloidal solution and through which rays pass (c) a photo-electric or thermo-electric cell connected to a millivoltmeter. The three parts of the assemblage are placed in a thermostat and changes in degree of transparency or translucency or opalescence are measured directly on the arbitrary millivoltmeter scale without.

As a *mathematical expression* for the degree of opacity or its converse, transparency, we have as follows:

In the plane-parallel layer, if a represents the relation of impinging light i to transmitted light i' $a = i'/i$ as an expression for the index of transparency of the solution. This is always a real fraction. For a perfectly transparent layer it equals one and for an absolutely opaque layer it is zero. The transparency of a containing series of layers equals the product of the individual transparencies.

In our experiments we used Elliott's thermoelectric cell consisting of a couple of iron-constantan of 0.1 mm. diameter. The first studies with this cell were to determine the limit of time exposure to the light which gave concordant results in several nephelometric experiments. The results are given in the table below.

TABLE XVII
THE CUUVETTE CONTAINING DISTILLED WATER AT 38° C.

Duration Between Two Experiments	The Cell Exposed to the Light During			
	12 Seconds		20 Seconds	
0	100	—8.5	168	8.4
5	100	8.5	170	8.5
10	100	8.5	169	8.45
20	100	8.5	168	8.4
30	100	8.5	168	8.4

These results demonstrate that the thermoelectric cell gives sufficiently large galvanometric readings for small exposures to the light, values rigorously in proportion to the time of exposure. Several experiments were then carried out using coagulating systems prepared as above, in order to follow the changes in the degree of transparency during coagulation.

A striking parallelism is observed between the course of change in the diminution in transparency and the change in viscosity during clot-

TABLE XVIII
DIMINUTION IN THE INDEX TRANSPARENCY DURING COAGULATION

Solution Examined	Reading in 12 Seconds
Distilled water (standard) at 39° C.....	100
Fibrinogen thrombin initially.....	67
After 5 minutes	66
" 10 "	62
" 15 "	56
" 20 "	47
" 25 "	36
" 30 "	26
" 37 "	26
" 30 " clot	23
After syneresis of the clot (exuded serum).....	82

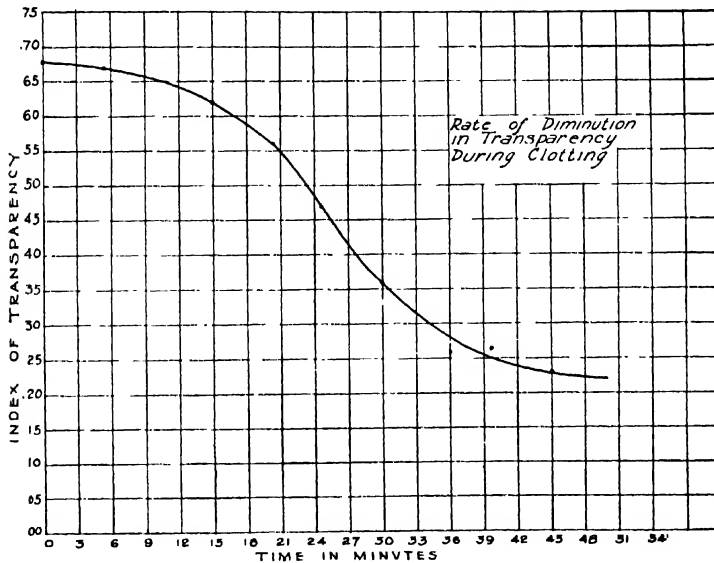


FIG. 6.

ting. The curve representing the changes in transparency is autocatalytic and on strict comparison with the viscosity curve is quite analogous to it. Further, the exuded serum is optically clearer than the initial mixture. Evidently after syneresis separation of the clot from the system removes simultaneously some of the causes responsible for opalescence.

The diminution in the degree of transparence and the increase in opalescence in the clotting system may be interpreted on the same basis as in the viscosity changes above. In the slow latent period the changes in transparency are slight for (a) the increase in the size of the particles is correspondingly slight, (b) the increase in particles due to very fine salt precipitation together with dust particles acting as nuclei condense more layers of the dissolved substances and so increase the opalescence. In the rapid coagulation period the changes in transparency are correspondingly rapid in that (c) an increase in the heterogeneity of the dispersed medium takes place, (d) formation of a net-like reticulum structure occurs, (e) there is increase in the bulky water-complexes associated with each of the protein ions.

X. EFFECT OF TEMPERATURE ON THE RATE OF CLOTTING

Optimum clotting rate is so easily attained by elevation of the temperature of the system, keeping other conditions constant that a quantitative study of this physical factor became of interest. Clotting time determinations were made in the usual way and the results are given in the table below.

TABLE XIX
EFFECT OF TEMPERATURE ON RATE OF CLOTTING

Absolute Temperature	Clotting Time	
312	25 m.	13,944
303	35	13,950
293	30	13,924
273	200	13,924

Analysis of the results obtained showed that the temperature coefficient of the clotting rate was far too great to conform to the values and relations for homogeneous or to those of heterogeneous systems. For homogeneous systems, we have:

- (a) The lower the order of reaction, the higher the temperature coefficient.
- (b) The greater the reaction velocity the smaller the temperature coefficient.
- (c) The more a reaction is catalyzed the smaller the temperature coefficient.
- (d) The greater the concentration of a positive catalyst the smaller the temperature coefficient.

(e) The reaction velocity is doubled or trebled for a rise of 10 c.

For heterogeneous systems we have:

- (a) Rate of reaction is a sole function of diffusion rates.

- (b) Rate of reaction that is catalyzed is determined solely by the diffusion on rate of the catalyst surface.
- (c) Reaction velocity is increased about 1.2 for a 10° rise in temperature.

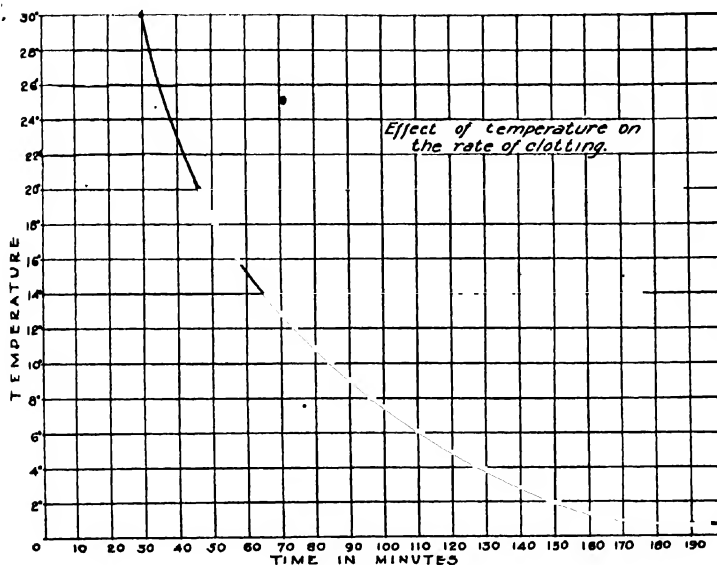


FIG. 7.

- (d) An empirical equation found by Arrhenius established a relation between effect of temperature on rates of reaction in irreversible systems.

$$\frac{K_1}{K_0} = e^{\frac{\mu}{2} \left(\frac{T_1 - T_0}{T_1 T_0} \right)}$$

Where e is the base of the Napierian logarithms, K_1 is the velocity of reaction at temperature T_1 and K_0 that at T_0 and μ is a constant. The greater μ is the more rapidly the velocity of reaction increases with temperature. This relation although not strictly deducible from the gas laws is analogous in form to the van't Hoff equation connecting the value of the equilibrium constant with the temperature and which is deducible for the gas laws.

Instead of measuring the velocity of reaction by means of determinations at arbitrary stages of clotting it may also be determined by evalu-

ating the time necessary for reaching the end-value of the process or the clotting time. In this case the velocity of reaction is inversely proportional to the time necessary for the reaction so that

$$\frac{K_0}{K_1} = \frac{t_1}{t_0} \quad (1)$$

and the equation above becomes:

$$\frac{t_0}{t_1} = e^{\frac{\mu}{2} \left(\frac{T_1 - T_0}{T_1 T_0} \right)} \quad (2)$$

$$\mu = 2 \left(\frac{T_0 T_1}{T_1 - T_0} \right) \log \frac{t_0}{t_1} \quad (3)$$

Concordant values obtained for μ confirm the applicability of this law. A point of general interest is to compare the average value of μ in the table with others already determined. It appears that values for natural reaction associated with animal life are within this range.

TABLE XX

Emulsion of yolk by pancreatic juice.....	13,600
Respiration by plants.....	14,800
Cell division in eggs.....	14,100
Normal Blood Clotting.....	13,940

Raising the temperature in the clotting system increases the peptizing action and therefore the degree of dispersion of its components. Ultra-microscopic examination of the mixture of thrombin and fibrin kept separately at 39° C. indicated less visible particles than of the same solutions at ordinary temperature. In other words the greater degree of dispersion of the particles contributes to a maximum frequency of collisions. This Brownian Movement effect in the system eliminates the characteristic slow diffusion phenomena that proceed in ordinary heterogeneous solutions.

CONCLUSIONS

1. The coagulation of oxalated plasma or fibrinogen is accompanied by a diminution in the hydrogen ion concentration, rapid at first and then more slowly unto equilibrium attainment as a continuous phenomena. The higher the hydrogen ion concentration the greater is the amount of hydrogen ions adsorbed during coagulation, the average amount adsorbed being $50\% \pm 10$.

2. The optimum for coagulation is about pH 7, coinciding with the isoelectric point of fibrin (pH 7.2). Clot formation is slower on either side of neutrality but hydroxions of equivalent concentration as hydriions retard to a much greater extent.

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3. Clotting takes place only between pH 4.75 and pH 8, these limits defining the isoelectric points of seroglobulin (pH 4.55) and seroalbumin (pH 4.7) on the one side and that of fibrinogen (pH 8) on the other.

4. Excessive hydriions or hydroxions do not destroy the activity of the thrombin solution but rather retard or prevent (beyond the limits defined in (3)) the formation of fibrin filaments visible ultramicroscopically.

5. The electrical conductivity diminishes during coagulation. The decrease is first rapid and then slower approaching an asymptotic equilibrium limit. This type of relation is analogous to the decrease in hydriions and includes that, but the conductivity decrease is greater than could be equated from hydriion diminution and this difference has been found to be due to the loss in ionic calcium.

6. Excessive sodium and calcium ions, always components of clotting systems, affect the clotting time and syneresis. Excess calcions retard clotting to a greater extent than natrions. Excess calcions retard syneresis while excess natrions favor the process.

7. The calcion concentration is regulated by calcion buffers. They are electrolytes which resist the change in calcion concentration upon addition of calcium salts. Calcion buffers are mixtures of weak acids and their salts which react to form insoluble, normal calcium salts and soluble, intermediate calcium salts.

8. The calcion concentration of any calcion buffering solution is determined by the ratio of the concentrations of the free buffer acid, HA , to the buffer salt, BA , according to the relation,

$$Ca^{++} = K \frac{[HA]^n}{[BA]^{2n}}$$

where n is the ratio of the valence of calcium to that of the acid, and K is an equilibrium constant.

9. Calcion concentrations may be expressed in logarithmic units as $\log \frac{1}{[Ca^{++}]} = pCa$ which is given by the general equation,

$$pCa = pK + n \log \frac{[BA]^2}{[HA]}$$

10. The calcion pK is 4.2 at 38° for the carbonates as calcion buffers.

11. The unit for the calcion buffer value of a solution is the number of gram equivalents of calcium salt or acid necessary to change the calcion concentration one unit of pCa . This is expressed by the differential ratio $\frac{d[BA]}{dpCa}$ which defines the calcion buffer value at any given calcion concentration.

12. The general equation for the calcion buffer value ρ , is

$$\rho = \frac{d[BA]}{dpCa} = \frac{2.3}{n} \cdot \frac{K'a[C] \cdot [H^+]}{(K'a + [H^+])(K'a + 2[H^+])}$$

This equation defines the calcion buffer value of the carbonates for which n is unity and that of the phosphates for which n is two-thirds.

13. The calcion buffer value, at any given hydron concentration, is directly proportional to the total concentration of buffer acid or salt.

14. The calcion buffer value is independent of the nature of the weak acid provided it forms an insoluble, normal calcium salt.

15. The calcion buffer value of a mixture of calcion buffers is the sum of the separate calcion buffer values.

16. The maximum calcion buffer value is attained when there are 0.586 part of buffer salt and 0.414 part of free buffer acid.

17. The molal calcion buffer value at the maximum is given by the ratio $\frac{0.395}{n}$.

18. The pH at which the calcion buffer value is a maximum is given by,

$$pH = pK'a + \log \sqrt{2}$$

which is pH 6.30 for the carbonates and pH 7.00 for the phosphates.

19. The molal calcion buffer value at pH 7.35 is 0.111 or 28 per cent of the maximum for the carbonates and 0.265 or 45 per cent of the maximum for the phosphates.

20. The calcion buffer value of the carbonates of normal blood serum at pH 7.35 is 3.5×10^{-3} and that of the serum phosphates is 0.5×10^{-3} . The combined calcion buffer value of blood serum is 4.0×10^{-3} .

21. The protective power of the protein components of the clotting system is increased in the course of coagulation attaining a maximum on syneresis.

22. The rate of coagulation is directly determined by the concentration of one component in the clotting system—the serozyme—associated with the serum proteins in the thrombin solutions. The substance is catalytic in its behavior, highly dispersed and thermolabile.

23. The changes in viscosity during clotting are slight at first and at a definite point of inflection rise suddenly and markedly attaining a maximum when syneresis commences; viscosity now decreases rapidly to a minimum for the exuded serum. The viscosity time relation is autocatalytic.

24. The changes in the index of transparency during coagulation is analogous to those in viscosity. The nephelometer, a newly devised apparatus, was used to follow the optical changes in the course of clotting. This apparatus may be applied generally for determining degrees of dispersion of colloidal systems.

25. The transition from fibrinogen to fibrin is accompanied by an increase in colloidal stability, adsorbability and degree of dispersion of the medium.

26. Coagulation is an autocatalytic process consisting of a latent period of precoagulation relatively long and a coagulation period relatively short. The latent period holds for the clotting system as a hydrophile and is reversible. The clotting period commences sharply at the point of transition from the hydrophile to the hydrophobic solution which is irreversible.

27. During the slow latent period, the electronegative serozyme nuclei condense the electropositive fibrinogen micellæ on their surfaces thus forming a system of spherical units. Instability due to electrical discharge and contact by coalescence results in a continuous reticulum.

28. The rate of coagulation of plasma or fibrinogen is a reaction of the same order as are most biologic reactions.

29. Three physico-chemical methods were devised in the investigation of this problem: (1) the nephelometer to measure the degree of transparency of a colloidal system as a function of its degree of dispersion, (2) the torsion viscosimeter to measure true viscosities of clots, (3) the inverse ultrafilter for rapid micro quantitative ultrafiltration.

Yale University, School of Medicine,
New Haven, Conn.

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THE EFFECT OF ANIONS UPON THE PHYSICAL, CHEMICAL, AND COLLOIDAL PROPERTIES OF ALUMINIUM HYDROXIDE

BY LEWIS B. MILLER

The past quarter century has witnessed the development of the rapid sand filtration process for the purification of potable waters. This process requires the addition of a chemical coagulant. The coagulant most commonly used is filter alum (commercial aluminium sulfate). While considerable empirical information is available regarding the use of filter alum, little is known of the chemical aspects of the process. It is the purpose of this paper to discuss some of the fundamental principles involved in the use of aluminium compounds in water clarification.

If to a dilute solution of a soluble aluminium salt, such as aluminium sulfate, is added an alkali, a precipitate, commonly called aluminium hydroxide, is first formed. Upon further addition of alkali the precipitate redissolves. In other words there is an intermediate range of hydrogen ion concentration in which the precipitate is stable. Since it is this precipitate which appears to produce the clarifying effect in water purification, we shall deal with its properties, chemical, physical, and colloidal, and the factors affecting these properties in very dilute solution.

In the experimental work described in this paper, only very pure materials were used. C. P. chemicals were purified further by the usual methods. Solutions were made up in distilled water and standardized by the appropriate procedures. Since the purpose of the research is to discover fundamental principles underlying commercial water purification by alum, the solutions were as dilute as could be conveniently handled in the laboratory. As a preliminary step in this study there were made electrometric titration curves of several aluminium salts by sodium hydroxide. The work of Theriault and Clark (1923) who used this method of attacking the problem, was repeated and corroborated. These investigators have shown that when aluminium salts are titrated with sodium hydroxide a curve similar to that shown in Figure 1 results. They have pointed out that there is a decided increase in pH values before three equivalents of alkali have been added and have suggested as a possible explanation of this phenomenon that the precipitate is carrying down acidic constituents from solution. In their paper is given

an excellent discussion of the theoretical and experimental aspects involved in the application of the electrometric titration method to problems of this type.

Bearing in mind the suggestion of Theriault and Clark regarding the composition of the precipitate formed by addition of alkali to an aluminum salt, an attempt was made to determine the composition of the precipitate directly. (For a complete description and discussion of this work see Miller, 1923.) To liter quantities of a .005 molar solution of potassium alum were added varying quantities of sodium hydroxide.

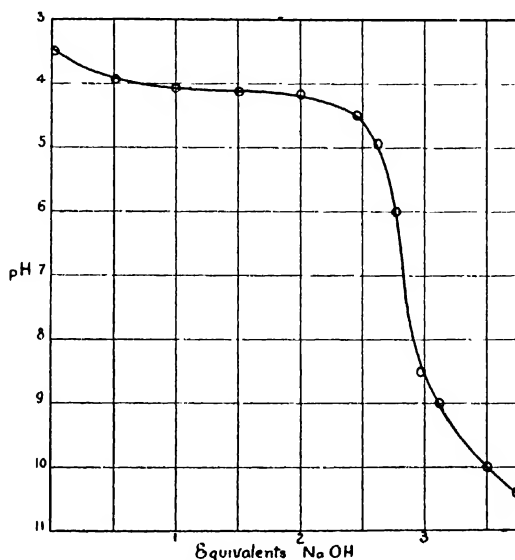


FIG. 1.—Electrometric titration of dilute alum solution by sodium hydroxide.

The pH value of the resulting mixture was determined colorimetrically (Clark, 1922). The precipitate was repeatedly washed with distilled water by alternately centrifuging and decanting it until the wash water no longer gave a test for sulfate. The precipitate was then analyzed for its aluminium and sulfate content. In Figure 2 is given a graphic representation of the results obtained by plotting the molar ratio of aluminium to sulphate found in the precipitate against the pH value of the corresponding solution. From the lowest pH value at which a precipitate forms up to a pH value of about 5.5 the composition of the precipitate remains constant and may be represented approximately by

the ratio 10, $\text{Al}:3(\text{SO}_4)$. At higher pH values the sulfate content of the solid phase decreases and becomes zero above a pH value of about 9.0.

If, as in Figure 3, the composition of the solid phase is plotted against the equivalents of sodium hydroxide added per mol of aluminium present, an interesting relationship is indicated. The composition of the precipitate remains constant until about 2.4 equivalents of alkali are added. When further quantities of alkali are added to the alum solution the sulfate content of the precipitate decreases, becoming zero when exactly three equivalents of alkali have been added. That is,

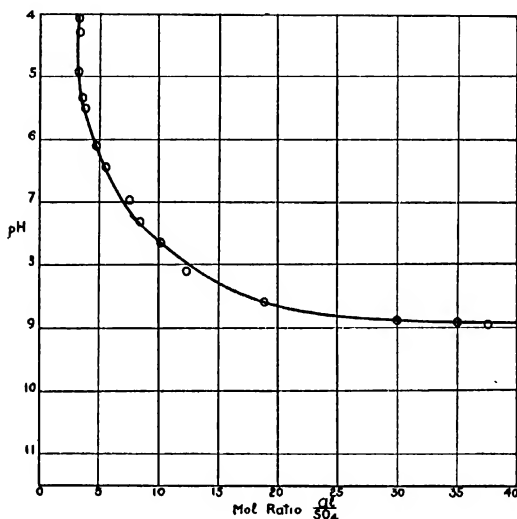
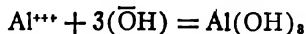


FIG. 2.—Composition of the precipitate from alum at varying hydrogen ion concentrations.

sulfate is present in the solid phase when less than the quantity of alkali necessary to react according to the equation



is added. When the alkali added is equal to or greater than the quantity necessary to react according to the above equation, no sulfate is found in the solid phase. That this represents a condition of equilibrium for the freshly prepared precipitate is indicated by the fact that the composition of the solid phase is the same in whatever order the reagents are added to each other.

It was likewise found that other polyvalent negative ions were carried down in the solid phase similarly to sulfate. It was never possible to demonstrate the presence of monovalent anions, such as chloride, in the solid phase due to the tendency of this phase to disperse colloiddally in the absence of polyvalent anions.

Charriou (1923) has observed the presence of anions in the well washed hydroxides of various metals and states that these anions may be displaced by washing with salt solutions containing anions of equal or greater valence. These observations were confirmed in our laboratory and led to a quantitative study of the displacement from the pre-

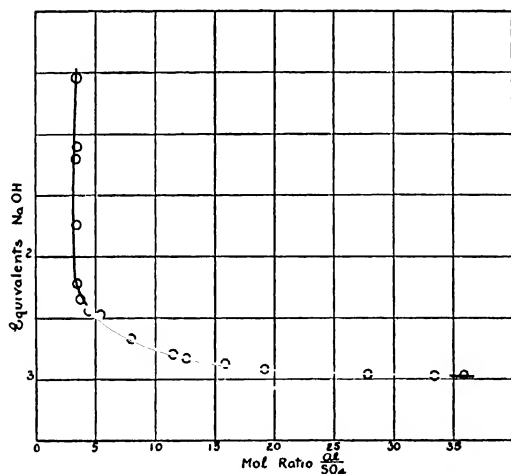


FIG. 3.—Composition of the precipitate formed by the addition to alum of varying proportions of sodium hydroxide.

cipitate of one anion by another. (For the complete description and discussion of this work see Miller, 1924.) Briefly, it was found that in the reciprocal displacement of, for example, sulfate and oxalate ions an equilibrium was always established in which the concentration of these radicles in the solid phase was determined by the ratio of their ionic concentration in solution. That is

$$\frac{[\overline{\text{SO}}_4]}{[\overline{\text{SO}}_4] \text{ solid}} = K_1 \text{ and } \frac{[\text{C}_2\overline{\text{O}}_4]}{[\text{C}_2\overline{\text{O}}_4] \text{ solid}} = K_2$$

From these experiments it was deduced that the precipitate consisted of two or more compounds in a single phase—a solid solution.

These observations upon the displacement of one anion by another were extended to the adsorption of dyes by the washed precipitate containing sulfate. It was found that, in general, basic dyes were adsorbed very little. Likewise, acid dyes containing only one moderately strong acid group (*e.g.*, methyl red) were adsorbed to only a slight extent. On the other hand dyes containing two or more strongly acid groups (*e.g.*, di, tri, and tetra potassium sulfonates of indigo) were taken up readily by shaking with the washed precipitate while at the same time sulfate was displaced.

It was qualitatively observed at this stage in the work that not only the composition of the solid phase but its physical and colloidal properties (especially with reference to its existence in the flocculated state or in the dispersed state) were at least partially dependent upon the concentration and valence of the anions present. Likewise, qualitative observations indicated that the range of hydrogen ion concentration over which formation of "alum flocc" occurred was influenced by the anions present. (In this work the term "alum flocc" has been applied to the complex insoluble substance of variable composition which separates when an alkali is added to a soluble aluminium compound, and which so obviously is not simply aluminium hydroxide.) Since purification of water by the use of alum is believed to be chiefly concerned with the formation of "alum flocc" these points were further investigated.

The range of hydrogen ion concentration over which flocculation occurred for solutions .005 molar with respect to aluminium ion in the presence of different anions was quantitatively determined. In Figure 4 are represented the data showing the effects of sulfate and chloride ions. These will suffice to indicate the principle. With alum practically complete precipitation occurs between pH values of about 5.3 and 8.7. With aluminium chloride complete precipitation occurs over a much narrower pH range—between approximately 7.8 and 8.6. It must be emphasized, however, that the pH ranges indicated for the chloride and sulphate ions are "flocculation" ranges and not "insolubility" or "isoelectric" ranges. To illustrate this point: In presence of sulfate ion coagulation or flocculation of the solid phase is always complete leaving the supernatant liquid, after the precipitate has settled, clear and sparkling. When alkali is added to aluminium chloride, however, the solution becomes opalescent and increases in opalescence as increasing quantities of alkali are added, the insoluble material forming a stable colloidal dispersion which cannot be removed by filtration nor by centrifuging in the ordinary laboratory centrifuge. When sufficient alkali has been added to raise the solution to the indicated pH value, flocculation occurs. Under the conditions just described in which an opalescent solution is formed the solid phase forms a colloidal dispersion which is apparently positively charged, for, upon the condition of a

small amount of a neutral salt containing a polyvalent anion, coagulation takes place.

When these experiments were extended to more dilute solutions, approaching in concentration those used in water works practice, the phenomena just described were also found to hold at these greater dilutions. Theriault and Clark (1923), however, in determining the pH range over which *rapid* flocculation occurred with very dilute alum solutions, found a very narrow zone to occur with maximum speed of flocculation near a pH of 5.5. It is interesting to note that this is in the most acid portion of the pH range of flocculation and in that portion

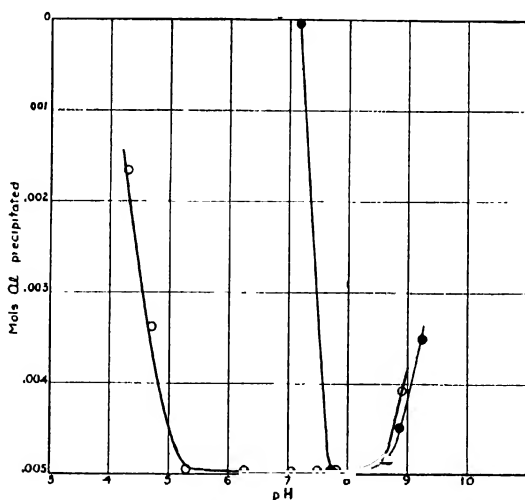


FIG. 4.—Zones of hydrogen ion concentration in which flocculation occurs for alum and aluminum chloride.

of the range where the solid phase is highest in sulfate content. In the present work it was observed that, in the more acid portions of the flocculation range, the precipitate was more dense, more rapid settling, more opaque, less gelatinous in appearance and less voluminous than that formed at higher pH values.

The studies upon aluminium compounds were also extended to the corresponding iron compounds which are likewise used in water clarification, although to a lesser extent. Allowing for the differences which must obviously occur in corresponding compounds of these two metals, the properties and principles which were described for "alum flocc" were found to apply equally to iron.

In this work it has been established that the precipitate which sep-

arates when an aluminium salt in dilute solution is treated with an alkali is not aluminium hydroxide (except perhaps at relatively high pH values) but a more complex substance containing varying proportions of those anions present in solution. To this complex substance has been applied the term "alum flocc." The dependence of the composition of the "floc" upon the hydrogen ion concentration has been indicated. The qualitative observations of other investigators upon the displacement of "adsorbed" anions from the aluminium precipitate has been extended and quantitatively investigated. The so-called "adsorption" of acid dyes by the aluminium precipitate has been shown under certain conditions to be closely related to this displacement phenomenon. In the displacement of one anion by another, an equilibrium is established in which the complex precipitate behaves as a single phase and partakes of the nature of a solid solution. In this respect the phenomena are very similar to the reciprocal displacement of cations in permutits. Hydrogen ion concentration, previously supposed to be the chief controlling factor in the formation of the aluminium precipitate, has been shown to be but one of the controlling factors. The valence and concentration of anion present in solution has been shown to be of equal importance.

This research indicates that in water purification by alum there are at least three chemical factors necessary for successful clarification: (1) There must be present a certain minimum quantity of aluminium ion; (2) there must be present an anion of strong coagulating power; and (3) the hydrogen ion concentration must be properly adjusted. Of all the anions studied the sulfate yields a "floc" with qualities best suited to successful water clarification. The range of hydrogen ion concentration over which flocculation occurs in presence of sulfate ion is broad. This "floc" is of good quality, rapid settling, and shows least tendency to form colloidal dispersions. (The salt, aluminium sulfate, from which it is formed is likewise well suited for its purpose,—being easily handled, very soluble and relatively cheap.)

The results obtained in this investigation may also serve to explain the reason for the variable results obtained by different investigators relative to the region of hydrogen ion concentration over which flocculation occurs in water purification and to the region in which considerable "soluble aluminium" is found in filter effluents. The cause in all probability lies partially, at least, in the effect of the negative ions present in the raw water or which are subsequently added. Similarly the power to control the formation of a precipitate in one pH range or prevent it in another by a variation of anion, or the knowledge that the removal of interfering anions will improve the character of the precipitate, must find application not only in certain phases of water purification but more especially in other industries which make use of precipitates of this nature.

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I wish to express to Professor Wm. Mansfield Clark, Chief of the Division of Chemistry, Hygienic Laboratory, my appreciation for numerous suggestions during the course of this work and for valuable assistance and criticism in the preparation of this manuscript.

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Hygienic Laboratory,
United States Public Health Service,
Washington, D. C.

NATURE OF THE COLLOIDAL SOIL MATERIAL

P. L. GILE

Literature dealing with the nature of soil colloids is too extensive and at the same time too fragmentary to be reviewed in detail here. Attention may be called, however, to some ideas that have been current.

Many of the earlier conceptions of the colloidal soil material were based on the behavior of the whole soil and were evidently prompted by the necessity of postulating the existence of something besides the ordinary soil minerals in order to explain the properties of the soil. Such views were speculations, but some of them were not so wide of the mark. The idea that the colloidal material of the soil is merely, or chiefly, a mixture of the common soil-forming minerals in an extreme state of subdivision has been held by many. The contrary view, that the colloid is a definite compound, has also been popular. We are chiefly indebted to Van Bemmelen (*6*)¹ for the idea, now generally accepted, that the colloidal material is similar in general nature to the synthetic inorganic gels that he studied so thoroughly.

Much of the earlier and some of the recent work on the subject could be criticized on the grounds that the data were obtained on the whole soil rather than on the colloidal material itself.

The purpose of this paper is more to present data that are suggestive of the nature of the colloidal soil material than to present a definite theory. The data used were obtained by various investigators in the Bureau of Soils in connection with special studies, some of which have already been published.

All determinations were made on colloidal material that had been isolated from the rest of the soil. Methods used in isolating the colloid have been described in a previous publication (*4*, p. 8 and 16). The colloids, suspended in distilled water, or in water containing just enough ammonia to impart a pH of 7 to 8, were separated from coarse particles by passing through a supercentrifuge. The maximum force to which a particle was exposed in the supercentrifuge was about 17,000 gravity for a period of three minutes. The colloid which passed through the centrifuge was collected on the outside of a Pasteur Chamberland filter by removing water by suction. The upper limit of the

¹ Reference is made by number (*italic*) to "Literature Cited," p. 227.

diameter of the dispersed particles was apparently about .3 micron and the average diameter was about .1 to .15 micron.

Chemical Composition

The colloidal soil material is made up chiefly of silica, alumina, iron, organic matter, and so-called "combined" water, that is, water not driven off at 110° C. Many other elements are always present, usually in much smaller quantity. Arranged in order of the quantity in which these usually are present, the greatest first, they are: magnesium, potassium, calcium, titanium, sodium, phosphorus and manganese.

Analyses of colloidal materials obtained from ten different soils are shown in Table I. These analyses, made by various investigators in the Bureau, were selected from a list of seventy in order to illustrate some of the differences that have been encountered in colloids isolated from different soils. Most of the soils represented are important agricultural types.

These analyses illustrate the wide variations that may occur in the colloidal material of different soils, without taking into account extreme soil types. Obviously, any theory of the nature of the material, based on the constancy of composition, cannot be applied to soil colloids in general.

However, the colloidal materials of soils in similar climatic regions may be very much alike in composition. Instances of this constancy have been pointed out by Robinson and Holmes (5, p. 15), and the recent analyses of ten Missouri colloids by Bradfield (3) afford an added example. The similarity in colloids from different soils which some investigators have encountered has apparently led many to believe that the colloid is made up chiefly of some one definite compound.

It is apparent from any one of the analyses that the colloidal soil material is decidedly a mixture. Ten elements are present in determinable quantities, in addition to organic matter and water. Probably it would be easier to construct a picture of the nature of the material if the elements present in small quantities were treated in a Pecksniffian manner and dismissed as "impurities." This seems hardly justifiable, however, since the minor elements, such as calcium, magnesium, titanium, potassium, etc., are present in all the colloids. Moreover, in certain colloids some one of the minor elements is present in appreciable quantity. The Aragon colloid, for instance, contains 3.54% titanium oxide, the Fallon colloid 5.32% magnesia, an Orangeburg colloid 0.6% manganous oxide, and a Dunkirk colloid 3.71% potash.

Treatments of different soil colloids with 1% sodium hydroxide, 1% and 15% hydrochloric acid and with 1% oxalic acid gave no evidence that a definite compound is the chief constituent of the

TABLE I
CHEMICAL COMPOSITION OF COLLOIDAL MATERIAL ISOLATED FROM VARIOUS SOIL TYPES
(Analyses by Messrs. Robinson, Holmes, Mattson, and Denison, Bureau of Soils, U. S. D. A.)

Soil Type	Locality	SiO ₂		TiO ₂		Al ₂ O ₃		Fe ₂ O ₃		MnO		CaO		MgO		K ₂ O		Na ₂ O		P ₂ O ₅		Organic Matter		Com- bined H ₂ O	
		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent		Per Cent	
Fallon loam, soil.....	Nevada.....	50.49	0.51	16.73	10.77	0.121	5.32	2.24	0.37	1.79	8.26	0.37	1.79	8.26	0.37	1.79	8.26	0.37	1.79	8.26	0.37	1.79	8.26	0.37	1.79
Sharkey clay, soil.....	Mississippi.....	50.13	0.46	21.70	8.70	0.035	2.54	1.86	0.69	3.83	8.73	0.69	3.83	8.73	0.69	3.83	8.73	0.69	3.83	8.73	0.69	3.83	8.73	0.69	3.83
Stockton clay adobe, subsoil.....	California.....	49.50	1.14	22.51	10.57	0.023	2.68	0.26	0.06	1.20	10.75	0.06	1.20	10.75	0.06	1.20	10.75	0.06	1.20	10.75	0.06	1.20	10.75	0.06	1.20
Marshall silt loam, soil.....	Nebraska.....	44.94	0.47	22.15	8.91	0.126	1.95	2.07	0.19	7.94	8.92	0.19	7.94	8.92	0.19	7.94	8.92	0.19	7.94	8.92	0.19	7.94	8.92	0.19	7.94
Carrington loam, subsoil.....	Iowa.....	48.04	0.65	25.19	8.80	0.032	1.53	0.89	0.38	4.52	9.23	0.38	4.52	9.23	0.38	4.52	9.23	0.38	4.52	9.23	0.38	4.52	9.23	0.38	4.52
Ontario loam, subsoil.....	New York.....	42.40	0.56	24.71	15.27	0.138	2.59	2.39	0.51	3.49	7.22	0.51	3.49	7.22	0.51	3.49	7.22	0.51	3.49	7.22	0.51	3.49	7.22	0.51	3.49
Sassafras silt loam, subsoil.....	Maryland.....	41.14	0.70	29.26	12.73	0.031	1.07	1.35	0.42	1.59	12.49	0.42	1.59	12.49	0.42	1.35	1.35	0.42	1.59	12.49	0.42	1.59	12.49	0.42	1.59
Sassafras silt loam, soil.....	Maryland.....	39.24	0.63	28.64	10.19	0.123	1.16	1.17	0.38	6.32	10.97	0.38	6.32	10.97	0.38	1.17	1.17	0.38	6.32	10.97	0.38	6.32	10.97	0.38	6.32
Vega Baja clay loam, soil.....	Porto Rico.....	36.26	0.65	32.85	12.44	0.160	0.18	0.36	0.47	4.15	12.73	0.47	4.15	12.73	0.47	0.36	0.36	0.47	4.15	12.73	0.47	4.15	12.73	0.47	4.15
Cecil loamy fine sand, soil.....	Georgia.....	31.30	1.01	33.64	11.66	0.07	0.78	1.71	0.58	6.33	11.79	0.58	6.33	11.79	0.58	1.71	1.71	0.58	6.33	11.79	0.58	6.33	11.79	0.58	6.33
Aragon clay, deep subsoil.....	Costa Rica.....	15.86	3.54	34.38	22.67	0.068	0.29	0.27	0.33	5.96	15.63	0.33	5.96	15.63	0.29	0.27	0.27	0.33	5.96	15.63	0.33	5.96	15.63	0.33	5.96

material. However, the possibility of such a compound being present was not precluded by the results. Material brought into solution by these treatments was on the whole no simpler or more definite in composition than the original material, and the same was true of the undecomposed residues.

At least part of the iron in the colloid seems to be present as an oxide, since dilute acids discharge the red or yellow color of certain colloids while dissolving part of the iron.

Nearly all the calcium, part of the magnesium, and part of the potassium seem to be present in the colloid in a somewhat different condition than the rest of the material. Robinson and Holmes (5) found that 1% acid dissolved all the calcium from several colloids but only portions of the magnesium and potassium. Unpublished data of Mattson and Anderson show that when soil colloids are treated with neutral salt solutions, with .05 N hydrochloric acid or are subjected to electro-dialysis, practically all calcium is removed, but usually only 10 to 15% of the potassium or magnesium, and mere traces of the other constituents.

As to whether the silica and alumina in the colloid are combined as an alumino-silicate, are present as oxides, or form secondary valence compounds, we have no conclusive evidence. Obviously, no one fixed proportion of silica to alumina would account for all the alumina or all the silica in every colloid, because of the variable proportions in which they occur. The probabilities for and against such a combination have been so frequently discussed in connection with permutite and the weathered products of minerals that it is unnecessary to review them here. Probably this question will eventually be answered by X-ray analysis.

Dr. Wyckoff of the Geophysical Laboratory, Carnegie Institution, kindly examined three widely differing soil colloids and obtained a distinct X-ray spectrum from each. It is therefore evident that the colloid is not wholly amorphous, although it is uncertain how much or just what crystalline material is present.

Although the colloidal soil material contains many elements, the quantities of which vary widely in different colloids, there seems to be some slight degree of regularity in the variations. Magnesium and potassium are usually both present in excess of calcium. This is in accord with the determinations, previously mentioned, which indicate that nearly all the calcium is loosely held in the colloid, but only a small part of the magnesium or potassium is in the same condition. There is a fair degree of correspondence between the relative proportions of silica, and iron and alumina in the colloid and the quantity of lime present. This is shown by the molecular ratios in Table II.

TABLE II
RELATION BETWEEN CONSTITUENTS IN DIFFERENT SOIL COLLOIDS

Source of Colloid	SiO ₂	CaO
	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{CaO}}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3} \times 10$
Fallon loam, Nevada.....	3.62	1.81
Sharkey clay, Mississippi.....	3.11	1.00
Stockton clay adobe, California.....	2.87	1.22
Marshall silt loam, Nebraska.....	2.73	0.73
Carrington loam subsoil, Iowa.....	2.64	0.75
Ontario loam subsoil, New York.....	2.08	0.63
Sassafras silt loam subsoil, Maryland.....	1.89	0.26
Sassafras silt loam, Maryland.....	1.85	0.38
Vega Baja soil, Porto Rico.....	1.34	0.17
Cecil	1.28	0.25
Aragon	0.54	0.18

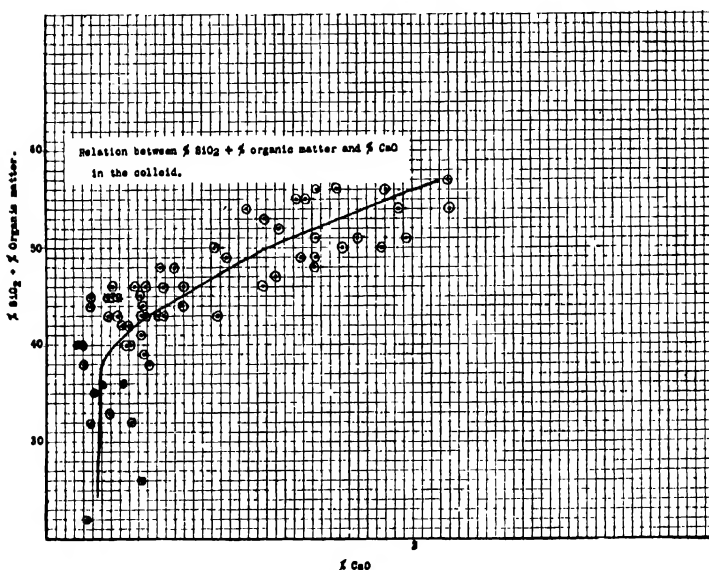


FIG. 1.

The relation between percentage of lime in the colloid and the combined percentages of silica and organic matter is shown for 70 different colloids in Figure 1:

It is apparent that the quantity of calcium, the chief replaceable base in the colloid, tends to vary with the $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio and with the combined silica and organic matter. This correspondence, however, is far from a strict proportionality.

Properties

Certain colloidal soil materials adsorb as much water vapor as Patrick's silica gel and have a heat of immersion in water as high as activated charcoal (1). Thus the soil colloids have some characteristics in common with materials which are generally considered to have a very fine porous structure.

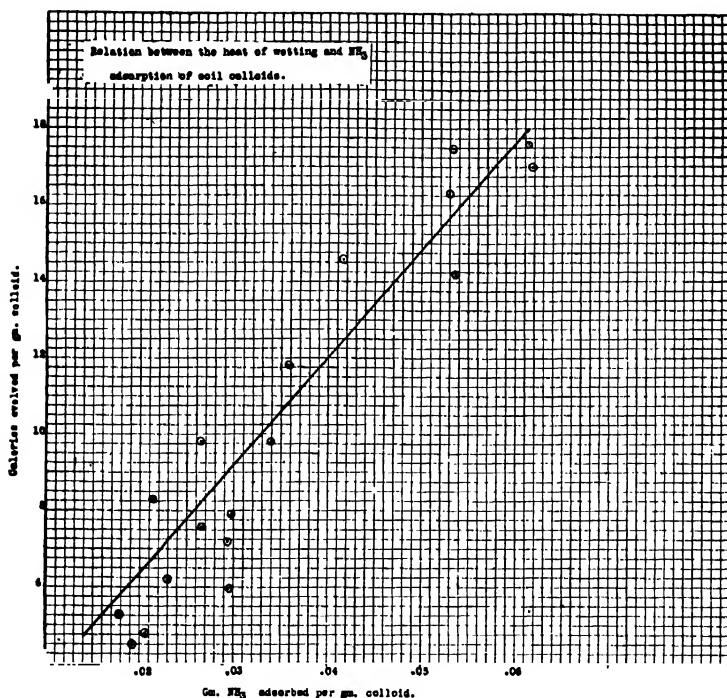


FIG. 2.

Soil colloids possessing a high adsorptive capacity of the "capillary" type usually show also a high "exchange" adsorption with neutral salts and basic dyes. Possibly this characteristic of having high adsorptive capacities of both types might be taken as distinguishing the soil colloidal material from many other colloids.

The colloidal materials from certain soils vary widely in the degree that they possess certain properties, judging by such determinations as: average size of dispersed particles, heat of wetting, swelling, viscosity,

volume of floc, influence of various substances on the cataphoretic movement, adsorptive capacity for water, ammonia, malachite green and neutral salts. Data of several of these determinations have already been published (2, 4) and data of the remainder will be published by Anderson and Mattson.

There is generally a correspondence between the response of a given colloid to one test and its response to another. A colloid, for instance, that has comparatively small dispersed particles, usually has a high capacity for adsorbing vapors, gives a high heat of wetting, swells markedly, occupies a large volume when flocculated and is comparatively resistant to neutralization of its electrical charge. In other words, if different colloidal soil materials are arranged in order on the basis of one determination, they usually fall in approximately the same or inverse order on the basis of another determination. There are exceptions to this parallelism. One soil colloid we have worked with seems to be habitually out of order.

An example of the relation between different properties of the colloid is shown in Figure 2. In this figure the heat of wetting of the colloid in water (expressed in small calories) is plotted against the quantity of ammonia vapor adsorbed.

It will be seen that the proportion between these two values is not exactly the same for all colloids, but it is fairly constant. Curves could also be plotted to show the proportionality between the other determinations mentioned. Some of the proportions would be as constant as that just shown; others would be more variable.

The fact that different colloidal soil materials tend to approximate a continuous, set order in a number of diverse determinations is taken as indicating that the different materials are all of the same general nature. And the fact that one or another colloid departs from the general order in certain determinations is taken as indicating a characteristic difference in the particular colloid.

Relation of Properties to Chemical Composition

The colloidal soil material contains so many elements in such variable proportions that a simple relationship between chemical composition and properties of the material would hardly be expected. It is somewhat surprising to find, however, that there is a fairly constant relation between the content of silica, alumina and iron in the different colloids and their properties. This is pointed out in a paper by Anderson and Mattson (2).

The proportionality between properties and molecular ratio of

$$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3} \text{ of a number of colloids extracted from different soils}$$

is shown in Figures 3, 4, and 5. Most of the data from which the curves were plotted has been published (1, 4, 5). In Figure 3 the heat evolved when the colloid is immersed in water is plotted against its $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio.

The relation between heat of wetting and $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio, is

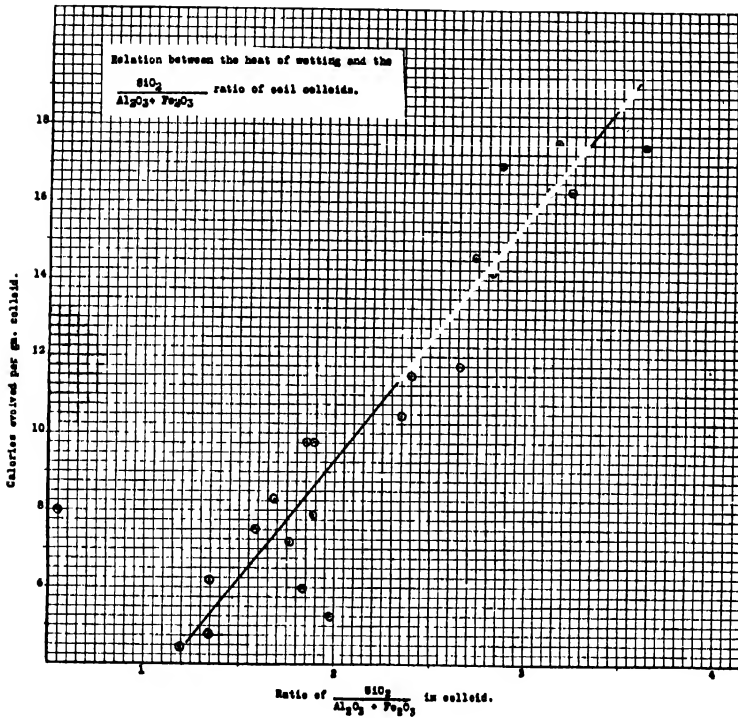


FIG. 3.

not exactly the same for all the colloids, but it tends to be fairly constant.

For colloids with $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratios varying between 1.20 and 3.60 the heat of wetting per gram approximates five times the silica ratio. This relation probably would not hold for colloids extracted from peat soils, and doubtless heat of wetting is not a straight line function of the silica ratio over the whole range of colloids in mineral soils. The eight

calories evolved by the Aragon colloid with a silica ratio of .54 suggests the possibility of the curve passing through a minimum.

In Figure 4 the quantity of dry ammonia vapor adsorbed by the oven dry colloid is plotted against the $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio.

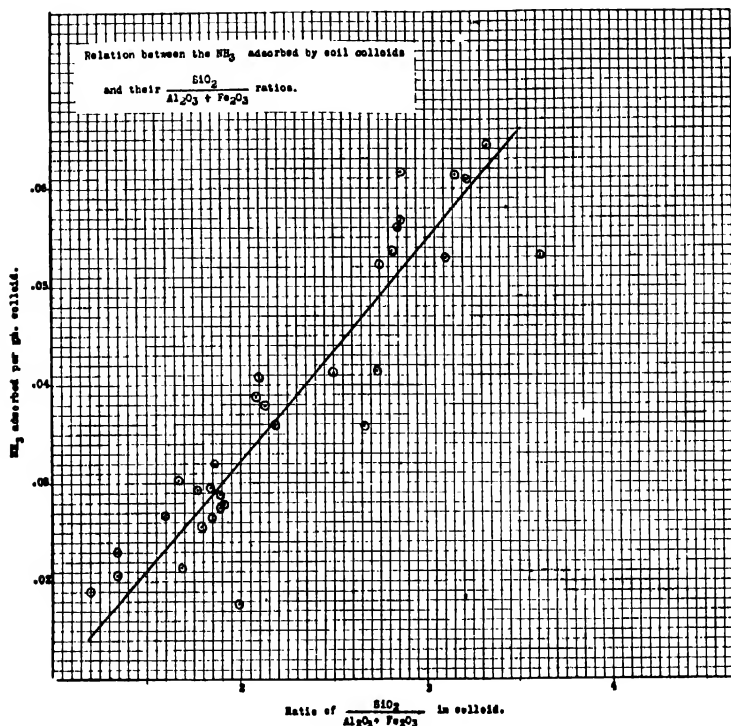


FIG. 4.

A few more colloids are represented in this graph than in the preceding; but they cover a narrower range in composition. In the case of these colloids the ammonia adsorbed per gram approximates $\frac{1}{60}$ of the $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio.

The proportion between quantity of malachite green adsorbed per gram of colloid and the $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio is shown in Figure 5.

The adsorption of malachite green seems to be a little less closely related to the silica ratio than is the heat of wetting or ammonia adsorption. Still there is a marked tendency to a proportionality.

Some of the other properties of the colloid which have been mentioned do not correlate with the silica ratio so well as heat of wetting, ammonia adsorption and adsorption of malachite green; but they all

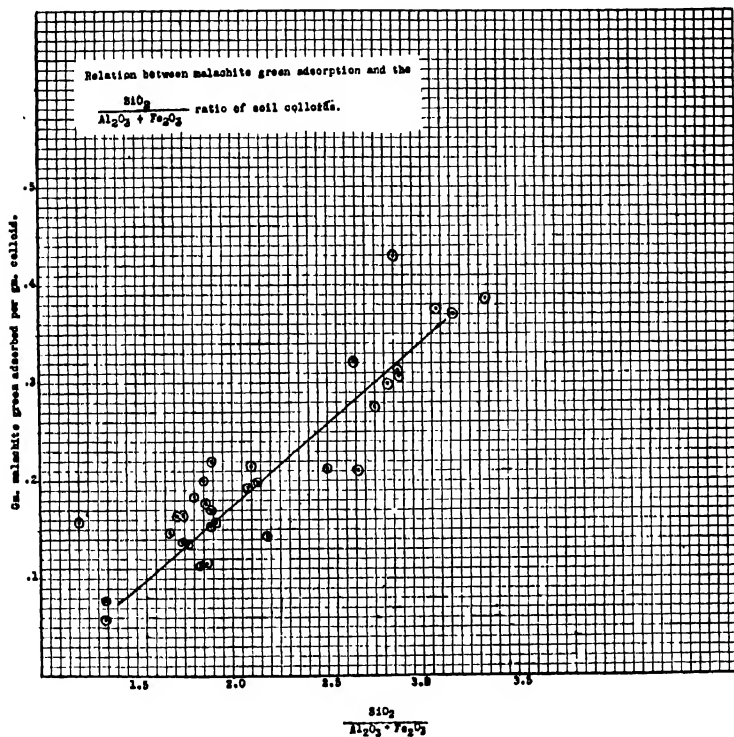


FIG. 5.

show some degree of relationship. A strict proportionality between properties and $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio would not be expected unless this ratio were the only, rather than the major, factor determining properties. Obviously, other constituents of the colloid which do not follow the $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ exactly must have some effect on properties.

Constitution of the Colloidal Material

A concept of the nature of the soil colloidal material should take into consideration the following facts:

The soil colloidal material contains ten or more elements in appreciable quantities besides combined water and organic matter. The proportions vary greatly in colloids from different soils. Silica, alumina and iron make up the greater part of the colloid in mineral soils. No conclusive evidence has been offered as to how the elements are combined; but X-ray analysis indicates the presence of crystalline material. Nearly all the calcium, a small part of the magnesium and potassium, and probably most of the sodium are present in a somewhat different condition from the rest of the material. The quantity of lime in the colloid shows a rough correspondence to the quantity of silica and organic matter present.

The different soil colloidal materials all seem to be of the same general nature, since they respond in approximately the same continuous order to a number of different determinations. The properties of the different colloids vary approximately as the molecular ratio of

$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ and to some extent as the replaceable bases. In most of the colloids calcium is the chief replaceable base, and it varies roughly with the content of silica plus organic matter.

On the basis of these facts, the soil colloidal material might be pictured as a complex mixture, made up of silicates, oxides, or both; and these constituents may be crystalline, amorphous, or both. This mixture of constituents possibly persists even in the particles into which the colloid can be dispersed. Since the dispersed particles are approximately one-tenth of a micron in diameter, some of the amorphous aggregates or crystals in the mixture would be very small.

According to this conception, a dispersed particle of the soil colloidal material would be like a loose mosaic made up of different kinds of stones of different sizes with an internal pore space. Silica and organic matter might be conceived of as scattered through the mosaic in such a way as to preserve the heterogeneity and to form most of the boundary walls exposed to a liquid. The replaceable bases would be held chiefly in the surface which silica and organic matter present to water, part of the basic ions diffusing into the liquid and forming a Helmholtz layer.

Perhaps the most that can be said for this picture is that it is vague enough not to contradict the facts. However, facts cannot be readily remembered without some scheme for associating them. We shall probably have a satisfactory concept of the colloidal soil material when colloid chemists get through with the structure of silica gel and gelatine and concentrate on mixed gels.

In many of the earlier ideas concerning the colloidal soil material sufficient importance is not attached to the fact that the colloidal soil material is a mixture. Possibly the existence of the colloidal material as a colloid is dependent on its being a mixture.

In most soils colloidal material has probably persisted several thousand years, undergoing some changes, but remaining nevertheless a dispersible colloid. The experiment will never be performed, so it is safe to predict that pure inorganic gels would not preserve their characteristics over the same period of time.

Possibly silica and organic matter are the constituents of the colloidal soil material which are chiefly responsible for the mixture remaining a dispersible colloid. When the sum of these constituents is reduced too far, certain other constituents of the colloid may pass out of the dispersible colloid state, through growth of crystals or induration of amorphous aggregates.

The composition of the Aragon soil suggested this idea. Approximately 16 per cent of the soil is made up of gibbsite (AlO_3H_3) in crystals large enough to be identifiable. Gibbsite is an uncommon soil mineral, and the colloidal material in the soil is unusual in having a very low $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$ ratio of .54. Possibly the gibbsite was formed by a segregation of alumina from the colloidal material, the segregation of alumina being dependent on a depletion of the colloidal material in silica and organic matter.

The formation of laterite in certain soils is probably accompanied by, if it is not dependent on, a removal of silica from the colloidal material. And possibly other indurated or crystalline materials are formed as a consequence of the colloidal material becoming depleted of other constituents.

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Division of Soil Chemistry,
Bureau of Soils,
U. S. Department of Agriculture
Washington, D. C.

THE COLLOID CHEMISTRY OF SOILS

By EMIL TRUOG

If a moist soil is examined with a hand lens or microscope, it will be found that the crystals of mineral matter, of which most soils are largely composed, are either partially or wholly covered with a coating of colloidal material. The colloids of the soil exist almost entirely in the gel condition, and a considerable portion of this gel material sticks to the grains of soil. The other portion of this gel material exists as separate aggregates of colloidal material. Two broad divisions of soil colloids may be made, namely, the organic and the inorganic. The organic colloids are dark in color, while the mineral colloids are light yellow to brown and red in color due to the presence of iron compounds. The organic and mineral colloids usually become mixed to some extent giving rise to variously colored mixtures, which determine quite largely the color of the soil.

Formation and Chemical Nature of Soil Colloids

The mineral colloids of the soil arise from the chemical weathering of the crystalline mineral matter. This weathering consists largely of hydrolysis and carbonation of silicates, resulting in the solution and leaching of salts of sodium, potassium, calcium, and magnesium and in the production of a comparatively insoluble colloidal residue consisting of acid silicates, acid alumino-silicates, and ferric, aluminum, titanium, and silicon oxides in various states of hydration. The excellent work of Bradfield¹ indicates that a considerable portion of the colloidal material of soils consists of complex acid alumino-silicates rather than a mixture of the separate colloidal oxides. These colloids are probably formed to a considerable extent at the surfaces of crystals and either remain sticking on these surfaces or form separate aggregates.

The organic colloids of the soil are largely of plant origin, and are introduced into the soil in the form of plant roots, stubble, other plant residues, and animal manure. Soon after being introduced into the soil, this colloidal material undergoes profound changes due largely to the action of bacteria and other lower forms of life. The nature of the changes which go on is dependent on the conditions of aeration. Under

¹ "Colloid Symposium Monograph," 1, 369 (1928); *Jour. Am. Soc. Agr.*, 17, 253 (1926).

conditions of good aeration, called aerobic, such as obtains in well drained and cultivated soils, the organic matter is rather rapidly and completely oxidized, much as if it were burned, resulting in the formation of water, carbon dioxide, and the nitrates, sulfates, carbonates, and phosphates of calcium, magnesium, potassium and sodium. All these are made available for new plant growth, and there remains no trace of the organic matter.

Under conditions of poor aeration, called anaerobic, such as obtains in poorly drained marshes and swamps, the organic matter undergoes an entirely different kind of decomposition, from that just described, which results in the formation of at least one product which is quite different from any of those just mentioned. This product is still organic in nature and goes under the general name of humic acids, and consists of a black, waxy, colloidal substance which is a mixture of many complex acids and other compounds, as shown by the work of Schreiner and Shorey.² Chemically it is high in carbon and contains in addition oxygen, hydrogen, and nitrogen with varying admixtures of small amounts of practically all the elements found in soils. Humic acids may, in a way, be considered as representing the first stage in coal formation. If the humic acids are subjected to further anaerobic conditions and additional pressure, there results a product of higher and higher carbon content due to elimination of other elements and there is produced successively lignite and coal.

Aside from being found in marshes and swamps, humic acids are formed quite extensively in prairie soils which are covered with a dense growth of prairie grass. This grass forms a sod which prevents rapid aeration and provides, in a sense, partial anaerobic conditions which make possible the formation of humic acids from the dead roots, stems and leaves of the grass. The dark color of prairie soils is due to these colloidal humic acids or humified organic matter, which largely coats the particles of mineral matter. The humic acids, when once formed, resist decomposition even when subjected to aerobic conditions. This is evidenced by the fact that black prairie soils remain black many years after being brought under intensive cultivation.

The treatment which is most favorable under ordinary farming practice, for the formation of humic acids, is the seeding of the land to pasture and hay. Even under these treatments, the accumulation of humic acids is extremely slow, and if the land is in a rotation with grains and cultivated crops, there results practically no accumulation of humic acids and darkening of the soil.

The bacteria and other organisms of the soil may be considered as colloidal material of the soil, but they constitute, at most, only a relatively small portion. Assuming 5,000,000 bacteria per gram of soil,

² Bul. 74, 1910, Bureau of Soils, U. S. Dept. Agri.

with an average diameter of 1000 μ m and a sp. gr. of 1, the bacteria would constitute only .01% of the weight of the soil which is an extremely small proportion of the total organic matter usually present.

The amount of colloidal material in different soils varies very much. Assuming all particles of less than 1 micron in diameter to be colloidal, Gile* and co-workers of the U. S. Bureau of Soils, through their excellent work, have shown that the common loam soils may contain from 15 to 25% of colloids, and the clays 40 to 50% and even up to 90% and more. Sandy soils of course usually contain only a few per cent of colloids. The colloidal matter of the soils just mentioned is usually largely mineral, and rarely is 10% or more of it organic. Peats, however, are made up quite largely of organic matter which is colloidal. Black prairie soils may contain 15 to 20% of organic matter, while light colored loams usually contain 5 to 10%. Most of this organic matter is colloidal.

Colloids Multiply the Internal Surface of Soils

The part which soil colloids play in making a soil a favorable place for plants to grow is an extremely important one, as will be indicated shortly. The significant property of soil colloids, as of all colloids, is of course their enormous specific surface. The proportion of the total surface exposed in soils which may be attributed to the colloids present is really astounding.

The average size of the soil particle in sandy soils may be assumed to be .2 mm. or 200,000 μ m. If some of this material is changed to colloidal material whose particles have an average diameter of 100 μ m, the surface of the material changed is increased 2000 times. In other words, it is possible to have a sandy soil which contains only 1% of colloids and 99% of other material, and yet, approximately 95% of the surface exposed will be due to the 1% of colloids present and only 5% to all the rest of the material.

For purposes of another example, a silt loam, which represents the most valuable class of general agricultural soil, may be considered in a similar manner. The average size of particle in a silt loam may be assumed to be 20,000 μ m. If some of this material is changed to colloidal material whose particles have an average diameter of 100 μ m, the surface of the material affected will be increased 200 times. In other words, it is possible to have a silt loam soil which contains only 1% of colloids and 99% of other material, and yet, the 1% of colloids will give to the soil approximately two times as much surface as all the other 99% of material put together. Ordinarily silt loam soils contain

* Bul. 1193, 1924, U. S. Dept. Agri.; Bul. 1311, 1924, U. S. Dept. Agri.; *Jour. Am. Soc. Agron.*, 17, 270-275 (1925).

about 20% of colloidal material and this material gives to the soils approximately 98% of the surface exposed, leaving only 2% to be credited to the 80% of other material.

In each cubic inch of ordinary loam soil there is probably exposed 25 or more square rods of surface and it is probably safe to say that 95 to 99% of this enormous surface which soils expose is due to the colloids present. The relation of size of particle to surface exposed and other properties is summarized in Chart I.

The Function of Soil Colloids

The part which soil colloids play in making the soil a favorable place for the growth of plants is an extremely important one, due to the controlling influence which surface exposure has on many factors that affect plant growth. Physically, the colloidal material acts as a cement which under proper conditions binds the particles of soil together in the form of a granular structure and prevents them from being blown or washed away at certain times. This provides for aeration and gives permanency to the home of the plant. Another factor of great importance is that the colloids greatly increase the water holding capacity of soils. This is of extreme importance in sandy soils. It is well known that the introduction of several per cent of colloids in the form of organic matter, which can be accomplished by good farming methods, greatly increases the water holding capacity of sandy soils, whose greatest drawback is usually their low capacity in this respect.

Alexander ⁴ has suggested the possible use of bentonite to increase the colloidal properties of soils. Since it would probably take 5 to 10 tons per acre to cause an appreciable effect, the cost would usually be prohibitive. Where deposits of clay exist near sandy land, it may prove profitable to haul and apply the clay on the land. This practice has been tried to a limited extent, and is much more apt to be profitable in case the land is located so that it may be used for intensive cropping rather than general farming. Under general farming conditions with relatively cheap land values, increases in the colloid content of soils can best be accomplished by adopting methods of farming which bring about an increase in the organic matter content of the soil.

Parker working at Wisconsin has shown that the colloidal material of soils as well as colloidal material in general lowers the freezing point of the most closely held capillary and hygroscopic water. This may be of importance to soil bacteria which cling closely to soil particles, in preventing complete desiccation of the soil at low temperatures.

⁴ "Colloid Symposium Monograph," Vol. 2 (1924), p. 99.

[illegible]

Limit of microscope (ultra violet light).....	100 μ	Diameter pores of hardened filter paper	1,500 μ to 2,200 μ
Limit of ultramicroscope.....	10 μ	Diameter pores Chamberland filter....	200 μ to 400 μ
Limit of ultrafilter.....	1 μ	Diameter of bacteria.....	500 μ to 1,200 μ
Brownian movement starts at.....	5,000 μ	Diameter 200 mesh particles.....	74,000 μ

A cube 1 cm. on side divided to cubes 1 μ on side gives a surface of $1\frac{1}{2}$ acres.

CHART I

Complete desiccation in times of extreme drought is also prevented, largely by colloids, which again may be an important factor in the existence of many bacteria which are essential for a fertile soil. In fact, the colloidal gels of the soil furnish well regulated homes, physically and chemically, for the bacteria.

The water retaining power of the soil colloids is of great importance chemically, since water is required in practically all the chemical reactions which go on in soils.

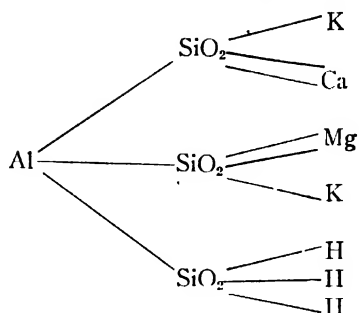
Chemically, the soil colloids determine quite largely the rate at which chemical reactions may go on in the soil, for Wenzel's Law states—"the reaction velocity of solids with liquids is proportional to the area of contact." In other words, applying this to the soil, the rate at which mineral elements dissolve in the soil solution and are made available for plant growth is proportional to the surface exposed by the soil particles. Since colloids diffuse and osmose little or not at all and would in fact be held back by the gels of the soil, plants cannot absorb food material in the colloidal condition, and are dependent entirely for their supply of mineral elements on what there is dissolved in the soil solution.

The common agricultural plants secrete from their roots practically no other acid than carbonic and this additional carbonic acid probably increases the rate of solution but slightly due to the large amount of carbonic acid normally produced by the soil bacteria, and to the highly buffered condition of most soils. Differences in the feeding power of plants for the more insoluble minerals are probably due largely to differences in ability to feed on dilute solutions, and absorption of the various essential elements in different proportions which affects the equilibrium conditions involved in solution, more favorably in some cases than others.

How soil colloids, in a sense, regulate the use and conserve the supply of essential elements which plants must obtain from the soil can probably best be explained by a consideration of the elements nitrogen, phosphorus, and potassium. These are the elements usually supplied in commercial fertilizers due to a frequent insufficiency of them in the soil for normal growth. Before considering these elements in detail it will probably be best to give some further consideration to the chemical nature and properties of soil colloids.

The mineral colloids of the soil appear most likely to consist largely of acid aluminosilicates in various stages of neutralization depending on the supply of bases in the soil and may be represented^a as to type as follows:

^a "Colloid Symposium Monograph," Vol. 1 (1928), p. 289.



In addition they usually contain large admixtures of iron and aluminum oxides. Usually the soil colloids are higher in phosphorus, potassium, sulfur and magnesium than the rest of the unweathered soil material.

The soil colloids, taken as a whole, both organic and inorganic, are amphoteric in nature and exercise a tremendous buffer effect on the soil. This buffer effect stabilizes the soil solution as regards reaction and concentration of dissolved material in a manner exactly similar to the buffer effect of colloids in living plants and animals and other chemical systems.

Considering now specifically the relation of soil colloids to the element nitrogen as regards its conservation for use by plants, it may be stated as follows: The original source of nitrogen for plants other than the legumes, is the organic matter of the soil. Before this nitrogen can be used by plants it is changed by bacteria to ammonia and then nitrates. Nitrogen in the form of nitrate is preferred by most plants. All soil nitrates are, however, very soluble and are not fixed in the soil to any appreciable extent. If nitrogen once gets in the form of nitrate, it leaches out and is lost whenever the rainfall is sufficient to cause a flow of gravitational water. Should nitrates be formed much more rapidly than needed by plants, there would be a great loss in the humid region. Fortunately nature has provided for this situation in the following way:

The amino and amide nitrogen of organic matter is first changed to the ammonia form. In this form it has strong basic properties and quickly combines with acidic colloidal material to form relatively insoluble compounds in the same way that potassium or calcium do. The ammonia is held rather strongly in this combination, and is released through hydrolysis slowly and continuously to be changed to the nitrate form. Fresh organic matter in the form of animal manure, commercial fertilizers, or plant residues is attacked rapidly by soil bacteria in a well cultivated soil, and there is released quickly an enormous amount of

ammonia. The same is true when ammonium sulfate is applied as a fertilizer. Were this ammonia to remain in solution, it would in cases be toxic to plant growth, and were all of it free at once to be changed over to nitrate, there would result an enormous loss under conditions just stated. The acidic colloidal material, because of its enormous surface, quickly removes the excess of the ammonia from solution and then regulates its release as needed by plants. For reasons indicated, the application of nitrate fertilizers should be in small and more frequent doses.

The relation of soil colloids to the element phosphorus as regards its conservation and use by plants will now be considered. The original source of phosphorus in the soil is apatite. Through weathering the phosphoric acid is released. In case the soil is not too acid and there is present considerable calcium bicarbonate in the soil solution, it combines rather completely with calcium to form colloidal calcium phosphate. If the soil is more than slightly acid, the phosphoric acid probably forms, largely, colloidal phosphates of iron and aluminum. When phosphate in the form of acid phosphate is applied to soils, it quickly goes into solution in the soil water, but is also quickly precipitated out again in the colloidal forms just mentioned. The fate of the phosphorus applied in animal manures and crop residues is the same.

The concentration of phosphorus in the soil solution is rarely over 5 to 10 parts per million of solution, but this concentration is maintained rather independently of the moisture content and the feeding of plants. This concentration is ample for plant growth, due to the fact that it is maintained at all the points where plant feeding takes place due to the large surface exposed by the colloidal phosphates.⁹

When rock phosphate, which is usually ground to pass a 200-mesh sieve, is applied to soils, it is at first not very available due to the inadequate distribution. These 200-mesh particles are in reality large boulders compared to the colloidal particles of soils as reference to Chart I readily discloses. Under certain soil conditions, particularly the acid one, these rock phosphate particles go into solution and are reprecipitated out in colloidal form. This effects a colloidal distribution through which each particle of original rock phosphate probably forms in the neighborhood of 10^{15} particles.

As regards conservation and the use of phosphorus by plants, the colloids provide ideal buffer conditions under which there is, first, a constant supply of basic material to fix the phosphorus whenever considerable amounts are released in soluble form through certain additions; and, second, there is later available for plants, a rather constant supply in the soil solution.

The relation of the soil colloids to the supply of potassium available

⁹ In this connection see Gordon, "Colloid Symposium Monograph," Vol. 2 (1924), p. 114.

to plants is an interesting and important one. Mineral soils are usually well supplied with potassium but since most of this exists in comparatively coarse crystals of feldspar and mica, its rate of solution and availability is very slow. Through weathering the potassium in these minerals is gradually liberated and at least a portion combines with the colloidal silicates and then has a greatly increased rate of solution, although the final concentration may not be high. With potassium as with phosphorus, rate of solution is important since that determines whether or not the concentration is adequately maintained at the points where plant feeding takes place.

When potassium is applied in the form of soluble fertilizers, it goes into solution and then through the movement of the soil water it comes into contact with colloidal silicates and combines with them as just explained. In this way a 200 pound per acre application of potassium chloride may produce visible results on plant growth for several years. Were it not for the favorable combination which it makes it would be largely lost by leaching in the course of a season in the humid region. This 200 pound application may add much less than 1% of the potassium already in the soil, and yet, because of its precipitation in the colloidal condition, it is so much more usable by plants that it produces a profound effect on plant growth. When potassium is applied in animal manure and crop residues, it becomes soluble rapidly in the form of salts and is then precipitated out in the same favorable way as when applied in the form of potassium chloride or sulfate.

The relation of soil colloids to other elements is equally interesting. The combination of sodium with the soil colloids is rather unstable in contact with water and carbonic acid and hence sodium is rapidly leached out of the soil into the ocean. This matters little, however, since sodium is not needed by plants. Magnesium combines readily with the soil colloids where it is conserved for plant use. Calcium forms the base, which, apparently, has been delegated by nature to play the leading rôle in the regulation of the reaction of the soil solution. To make this possible, it is not held so tightly by the soil colloids as potassium and magnesium but probably more so than sodium. As a result considerable calcium is leached out and must be resupplied at intervals in the form of ground limestone or burned lime.

Colloids Stabilize and Regulate Soil Conditions

It has already been stated that soil colloids are buffers and thus stabilize and regulate conditions. For the essential plant food elements, colloids are, in a sense, a store room with an efficient store keeper. Elements are only released when the needs arise, and if new supplies are added, these are safely stored away for future needs.

More or less thoughtlessly, farmers return animal manure, straw, and various kinds of refuse to the soil in both large and small quantities. One would think that this might throw the soil greatly out of balance, and yet this is seldom the case. The soil digests the material and stores up the products largely in combination with the colloidal material. Toxic compounds, if produced, are usually either basic or acidic in nature, and the amphoteric nature of soil colloids as a whole provides for their removal before a toxic concentration in the soil solution is produced. The toxic substance, when no longer produced, is gradually released to the soil solution, after which it is either leached away or destroyed by further bacterial activity. In this way the colloidal material, acting as a buffer, is in a sense a "shock absorber."

Of great importance is the stabilization of the reaction of the soil solution. This is admirably provided for by the buffering effect of the soil colloids. The production of carbonic, nitric and sulfuric acids in the soil, as brought about by the action of bacteria on soil organic matter, usually affects the H-ion concentration of the soil solution but little due to the buffer effects of the soil colloids. Gradually the soil colloids lose much of their basic material and become more acid and less efficient buffers towards the soluble acids just mentioned. When this has gone too far, the soil is said to be too acid, and the condition is corrected through the introduction of lime.

Soil Acidity Due to Colloidal Acids

Much has been written regarding the nature and cause of soil acidity. Some hold that it is due to physical adsorption of bases and a consequent liberation of acids which dissolve in the soil solution. Others hold that it is due to relatively insoluble soil acids of the aluminosilicic acid and humic acid type which are soluble enough to give the soil solution an acid reaction. Bradfield⁷ has recently put forward considerable evidence in favor of this theory.

Salter and Morgan,⁸ somewhat less recently, presented evidence in support of the adsorption theory. They held that if soil acidity is due to relatively insoluble acids, then the ratio of soil to water which is used in making the suspension for the electrometric determination of the H-ion concentration should not affect the results, since there would be an excess of acids present at all soil-water ratios. Since the soil-water ratio apparently affected their results, they favored the adsorption theory at least in part.

Within the past year, Pierre and the writer⁹ have repeated some

⁷ *Jour. Phys. Chem.*, **28**, 170 (1924).

⁸ *Jour. Phys. Chem.*, **27**, 117 (1923).

⁹ Data secured by W. H. Pierre in a thesis presented at the University of Wisconsin in partial fulfillment for the degree of Doctor of Philosophy, June, 1925.

work of this kind. It was soon found that the electrometric method is unsuitable for work of this kind, in which it is necessary to make H-ion determinations of very slightly buffered suspensions and solutions such as are obtained with low soil-water ratios. The reason for this is that a slight diffusion of KCl from the connecting bridge, slight contamination of alkali from glassware, slight impurities in the hydrogen, presence of nitrates in the soil, and slightly contaminated or so-called

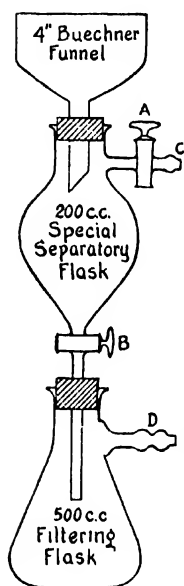


Fig. 1.—Illustration of a convenient ultrafilter devised by the writer. It is especially well adapted for filtering soil suspensions. The separatory funnel is specially constructed with a 1¼-inch mouth and a connection (C) for applying suction. To operate the apparatus proceed as follows: With the stopcocks (A) closed and (B) opened, suction is applied at (D) and a well fitting filter paper is placed on the funnel and thoroughly drawn down with water. The soil suspension to be filtered is then shaken and the funnel partly filled with it. Moderate suction is applied until the free liquid has passed through. The suspended material thus produces a uniform layer over the filter paper and subsequently acts as an ultrafilter for the remaining soil suspension. More of the soil suspension is added to the funnel and the stopcock (B) is closed occasionally to see if the liquid is filtering clear. When the liquid filters clear, the stopcock (B) is closed permanently and the suction is applied at (C), and (A) is opened. The essential feature of the apparatus is the special separatory funnel which makes it possible, without relieving the suction, to tell when the filtrate is coming through clear, and to catch the clear filtrate separate from the first filtrate, which always comes through cloudy. It is imperative, that, during the whole process, suction is never completely removed from the filter, otherwise colloidal material will creep under the filter paper and later come through; and also, that, the liquid never be so completely removed from the layer on the filter paper that this layer cracks and then allows colloidal material to pass through. In pouring soil suspension into the funnel, care should be taken so that the ultrafilter layer is not unduly disturbed. Since the ultrafilter is made of

the material to be filtered, adsorption and contamination by the filtering medium are prevented. This is an important feature in securing soil extracts for many purposes. The size of the various parts of the apparatus as given are convenient for ordinary work and may be changed to meet the needs of special work.

"poisoned electrodes" can easily affect the reaction of slightly buffered soil suspensions and solutions. Beans and Hammett¹⁰ have recently called attention to the difficulties involved and precautions necessary in working with slightly buffered solutions.

Because of these difficulties, it was decided to use the colorimetric method. In using this method it was found imperative to observe the following precautions:

1. The indicators must be neutral. If it is necessary to grind them

¹⁰ *Jour. Am. Chem. Soc.*, 47, 1215 (1925).

before solution, this should never be done in glass or porcelain mortars because of contamination with alkali. An agate mortar may be used.

2. The test tubes and glassware used must be thoroughly weathered. This is best done by heating for one-half hour in dichromate cleaning solution.

3. The solution to be tested must be perfectly clear. This is accomplished best by filtering with the apparatus shown in Figure 1. Thoroughly washed filter paper must be used. The centrifugal method is not nearly as successful for obtaining perfectly clear solutions, and if the method is used, the containing vessels must be stoppered during centrifuging, otherwise the acidity usually markedly decreases.

With these precautions in mind, the H-ion concentration of filtered soil extracts, made with soil-water ratios of 1 to 2, 1 to 20 and 1 to 50, were determined colorimetrically and the results are reported in Table I. In some cases the same result was secured regardless of the soil-water ratio. In other cases the H-ion decreased with a decreased proportion of soil. In the cases where this happened, fresh samples of soil were washed about 20 times with distilled water and the determinations repeated. The results obtained after this washing are also given in Table I, and they are now practically the same regardless of the soil-water ratio. Some soils contain a considerable amount of soluble salts which affect the H-ion concentration. With a change of soil-water ratio, the concentration of soluble salts changes and hence the effect on the H-ion changes. Soluble salts if present in considerable amounts must therefore be removed. Some soils probably also contain a larger proportion of rather soluble acids than others. These must also be removed.

After washing thoroughly, the effect of the soil-water ratio on the H-ion concentration of the relatively insoluble acids may be determined. The data presented show that within reasonable limits, the soil-water ratio does not affect the H-ion concentration and this supports the theory that soil acidity is due largely to relatively insoluble acids of the alumino-silicic acid type. Since some soluble salts like nitrates and sulfates are always present in the soil solution, small amounts of nitric and sulfuric acid are also present in the soil solution of acid soils due to the fact that these salts must be at equilibrium with the insoluble soil acids.

The question of soil acidity is intimately linked with the colloids of the soil. When the colloidal silicates, the acid silicates and the alumino-silicic acids, are largely neutralized due to an ample supply of bases, the soil solution cannot become acid due to the ease with which bases are secured from these colloids. When the colloidal acids, just mentioned, are less completely neutralized, bases are not secured so easily and as a result the nitric and sulfuric acid which are formed in the decomposition of organic matter may not become completely neutral-

THE H-ION CONCENTRATION OF SOILS AS AFFECTED BY THE SOIL-WATER RATIO AND PREVIOUS WASHING

Soil	Treatment	pH of Soil Extracts at Soil-Water Ratios of		
		1-2	1-20	1-50
Light brown silt loam.....	None	4.25	—*	4.8
	Washed	5.0	—	5.0
Reddish brown fine sandy loam..	None	4.95	5.25	5.45
“ “ “ “ ..	Washed	5.45	5.45	5.45
Black sandy loam.....	None	4.5	—	5.05
“ “ “ “ ..	Washed	5.1	5.2	5.2
Red clay	None	5.0	—	5.2
“ “ “ “ ..	Washed	5.4	—	5.4
Poorly decomposed peat	None	3.65	4.3	4.8
	Washed	5.2	—	5.3
Brown fine sandy loam.....	None	5.6	5.7	5.6
Black silt loam.....	“	5.55	5.55	5.55
“ “ “ “ ..	“	7.8	7.8	7.8
Gray fine sandy loam.....	“	5.65	5.65	5.65
Brown silt loam.....	“	6.1	—	6.1
Light brown silt loam.....	“	6.05	—	6.05

ized giving rise to an acid soil solution. The relatively insoluble colloidal soil acids, as has been shown, have a sufficient solubility to cause an acid reaction of the soil solution.

University of Wisconsin,
Madison, Wis.

THE POWER OF SOILS TO ABSORB WATER FROM AIR

BY F. J. ALWAY

"The power of soils to absorb water from air is much connected with fertility. . . . I have compared the absorbent power of many soils with respect to atmospheric moisture, and I have always found it greatest in the most fertile soils so that it affords one method of judging of the productiveness of land."

So wrote, one hundred and eleven years ago, Sir Humphry Davy (1), the pioneer in soil physics. In 1924 Dr. Milton Whitney, in an article on the Colloid Chemistry of the Soil (2), states that for the estimation of the amount of colloids in soils the Bureau of Soils in its recent work has used three principal methods: the absorption of water vapor, of ammonia gas and of malachite green. The power of soils to absorb water from air he considers proportional to their colloid content. The connection between colloid content and soil fertility, already dealt with at this meeting, is generally conceded. It would appear that the first of Davy's views is now fully substantiated. But why has it taken over a hundred years?

Fifty years ago both Schloesing (3) and Hilgard (4) recognized that the properties of many soils are much influenced by their content of colloidal clay, but until recently the amount of colloidal material present in most agricultural soils was estimated far too low, and it was generally stated in the literature that there is not over 1 or 2 per cent present (2). Now Gile and his co-workers of the Bureau of Soils have come to the conclusion that the colloidal material, assuming all soil particles less than 1 micron in diameter to be colloidal and including all the organic matter, varies from a trace to almost 100 per cent, with 40 to 50 per cent in clays in general, 20 to 25 per cent in loams, and less in coarser soils (5).

It has been generally recognized that the moisture relationships of soils are largely dependent upon their fineness of texture, commonly expressed in the terms of a mechanical analysis, by which the soil is separated into several groups of particles, designated "clay," "silt," and several sizes of sand. The "clay," which includes all particles less than 5 microns in diameter, was assumed to carry all the colloidal organic material in addition to the mineral particles with diameters between 1 and 5 microns, while the "silt" (5 to 50 microns) and "sands" fractions

were assumed to be free of colloidal material. Great numbers of such mechanical analyses of soils from all parts of the country have been reported by the Bureau of Soils during the past twenty years as well as many by various state experiment station workers.

Gile, Davis (6) and their co-workers in the Bureau of Soils, now find that in these mechanical analyses the dispersion of the colloid has been so incomplete that from 25 to 97 per cent of the "silt" fraction thus obtained may be made up of aggregates of colloidal material, while even in the "sands" fraction an appreciable amount of colloid may be included. Finding the mechanical separation of the colloid from the finer-grained mineral particles practically impossible they propose to modify this time-honored method of mechanical analysis to such an extent that it would be used only to get rid of the colloid and silt, leaving behind the sands, which are then separated by sieves into the several groups—very fine sand, fine sand, medium sand, coarse sand, and gravel. The amount of colloid would be estimated by the "ratio" method, to be dealt with later, and the percentage of silt found by deducting the sum of the percentages of colloid and sands from one hundred. In order to free the sands of colloid, special precautions, not observed in the hitherto practised method of mechanical analysis, would be taken. Thus the reliability of the determination of both colloid and silt fractions would depend upon this new ratio method, in which the absorption of water vapor by the soil is compared, in finer work, with its absorption by the colloidal material separated from the same soil, or, in what may be designated routine analyses, with the average absorption of the colloidal material separated from a large number of representative soils. Comparing the absorptive capacities of the colloidal material separated from over 30 representative soils from different parts of the United States, the Bureau of Soils investigators found that the extremes varied with malachite green as much as 600 per cent and with ammonia as much as 300 per cent, but with water by less than 50 per cent, or less than 20 per cent from the mean. They conclude that the mean absorptive capacity, 30 grams of water by 100 grams of soil colloid, may be used in estimating the colloidal content of all soils.

$$\frac{\text{Absorption per gram soil}}{\text{Absorption per gram colloid}} \times 100 = \text{per cent colloid in soil.}$$

The method of determination is comparatively simple (6). From 2 to 4 grams of the air-dried soil passed through a 100 mesh sieve is exposed in a wide shallow weighing bottle over 3.3 per cent sulphuric acid, in a vacuum desiccator exhausted to 50 mm. or less, the whole being kept for 48 hours in a thermostat with the temperature held constant at some point between 25° and 35° C. The amount of absorbed water is determined by drying by 110° C. for 18 hours.

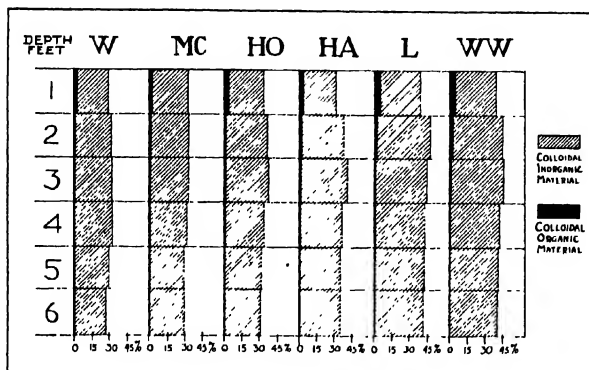


FIG. 1.

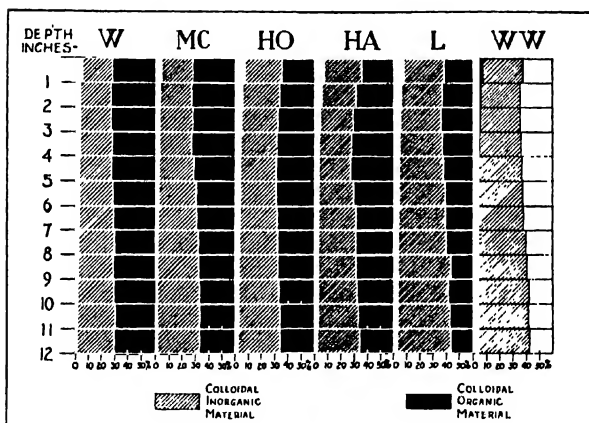


FIG. 2.

These figures were prepared from data of Alway and McDole in *Soil Science*, v. 1, pp. 216, 218, and 228 (1916). Six sets of soil samples from Wauneta (W.), McCook (MC), Holdredge (HO), Hastings (HA), Lincoln (L) and Weeping Water (WW).

Those few of you who have determined the hygroscopic coefficient of soils by Hilgard's original method will see in the above described determination of the absorptive capacity an improved and refined method for the determination of the hygroscopic coefficient, for another purpose to be sure, but one that will give very much the same values.

If the conclusions of the Bureau of Soils workers, that the absorption

of water by soils is due almost entirely to the colloidal portion, are sound, what a field is opened up for the discussion of soils matters from a colloidal standpoint. As the percentage of colloidal material will be about $3\frac{1}{3}$ times the hygroscopic coefficient, a few hours computation on our data of 10 or 15 years ago will give us a good idea of the colloidal content of hundreds of soils. In the case of the loess soils of the Transition Region, we at once have revealed to us the variation of the colloidal content from west to east through hundreds of miles and from the surface downward through 6 feet, (Fig. 1) and in the surface foot downward inch by inch (Fig. 2). The water retaining capacity of soils in the field, the water content of soils when plants rooted in them die, the availability of the water in soils, the downward movement of water (7), we will have at once in terms of the colloid content.

It is inadvisable to attempt any extended interpretation from a colloidal standpoint of my old investigations without first critically examining the fundamental assumption of the Bureau of Soils workers, viz. that the colloids in a soil are responsible for practically the whole of its absorptive capacity.

They have found that the removal of much colloidal material markedly reduces the absorptive capacity for water vapor, as well as for ammonia and malachite green (8). In the case of ordinary soils they find over 95 per cent of this to be located in the colloidal portion. On account of the difficulty, or even the practical impossibility, of freeing the "silt" fraction from colloidal material, they investigated the absorptive capacity of the fine material—between 1 micron and 50 microns in diameter—separated from 21 common soil minerals, corresponding to the "silt" fraction of soils. This they found to have an average absorption of less than 1 (0.95) per cent with a maximum for the 21 minerals of 3.08 per cent, in the case of limonite, and a minimum of 0.05 per cent with quartz. Values of 4.7 to 7.7 per cent were found for the silt fraction separated from four soils, with unusual precautions taken to remove the maximum amount of colloid. Six of the same common soil minerals were ground to colloidal dimensions and the finest material, freed of particles above 1 micron in diameter, was found to have an adsorptive capacity varying from 36 per cent for muscovite to 8.7 for quartz. Determinations were made of the absorptive capacity of several synthetic gels that had been air-dried and then ground to pass a 200-mesh sieve. The values for these varied from 31 per cent for ferric hydrate to 109 for aluminum silicate.

On heating soils to about 900° C. they found they lost their absorptive capacity for malachite green, although the size of the non-colloidal particles remained unaltered. I have not found any report on their work on the absorption of water vapor by ignited soils and I hesitate to assume that ignited soils will show only a negligible absorption.

POWER OF SOILS TO ABSORB WATER FROM AIR 245

Within the last few days I have had determinations made of the hygroscopic coefficients of ignited portions of a number of soils. About 12 years ago we had determined the hygroscopic coefficients of these same soils. We find that while the absorptive capacity is greatly reduced by ignition it still is 30 to 50 per cent of the original amount. (Table I.)

TABLE I
EFFECT OF IGNITION UPON THE HYGROSCOPIC COEFFICIENT

Soil	Source	Depth	Hygroscopic Coefficient		
			Before Ignition	After Ignition	Decrease
A	Southern Saskatchewan	1-6"	17.8	9.2	8.6
B	Western Nebraska	1-6"	10.5	4.7	5.8
C	Eastern Nebraska	1-6"	10.2	4.7	5.5
D	N.W. New Mexico	1-6"	10.0	5.9	4.1
E	Western Nebraska	1-6"	7.1	3.2	3.9
F	Western Nebraska	5'	5.6	3.0	2.6
G	Wauneta, Nebraska	1'	9.1	5.1	4.0
H	"	2'	9.6	4.8	4.8
I	"	3'	9.7	4.8	4.9
J	"	4'	9.9	5.1	4.8
K	"	5'	9.0	4.7	4.3
L	"	6'	8.3	6.1	2.2
M	McCook, "	1'	10.0	3.7	6.3
N	"	2'	10.9	4.9	6.0
O	"	3'	10.7	3.2	7.5
P	"	4'	9.7	5.8	3.9
Q	"	3'	9.1	4.9	4.2
R	"	6'	9.9	5.3	4.6

TABLE II
EFFECT OF IGNITION UPON THE MOISTURE EQUIVALENT

Soil No.	Source	Depth	Moisture Equivalent		
			Before Ignition	After Ignition	Decrease
1	Eastern Minnesota	1-6"	43.8	13.0	30.8
2	"	1-6"	49.6	21.3	28.3
3	"	3'	24.4	16.4	8.0
4	"	3'	25.0	14.7	10.3
5	"	6'	12.7	7.0	5.0
6	Eastern Nebraska	1"	31.5	12.2	19.3
7	"	3"	31.3	11.3	20.0
8	"	5"	31.7	10.4	21.3
9	"	8"	31.9	10.1	21.8
10	"	12"	32.3	11.8	20.5

During the past two years Pinckney and I have studied the effect of ignition upon the moisture equivalent of a large number of soils and

find that while the values are much reduced, 30 to 50 per cent in general, they still remain significant. (Table II.) Briggs and Shantz found that the moisture equivalent bears a linear relationship to the hygroscopic coefficient and the data of J. C. Russel and myself have confirmed this relationship. So we should expect the absorptive capacity for water vapor of all the finer textured soils that Pinckney and I have worked with to be fairly high after ignition.

In a very recent article Keen (9) with two of his fellow-workers at the Rothamsted laboratory report a detailed study of the absorption of water vapor by a soil both before and after ignition. They found that ignition lowered the absorption by about half.

It does not appear yet satisfactorily established that the ability of soils to absorb water vapor is a reliable measure of their colloid content.

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The University of Minnesota,
University Farm,
St. Paul, Minn.

MECHANISM OF LITHOPONE FORMATION

BY CHARLES A. MANN

Lithopone is one of the important white pigments used in paints, enamels, for linoleum and for compounding with rubber. It is pure white, very fine in texture, flocculent and non-crystalline,¹ and in a state of sub-division equal to that of white lead. It has the same tinctorial strength but more hiding power than pure zinc oxide. It is non-poisonous, has little or no basicity, and is stable in every medium known for paints except those of high acidity.² It is unaffected by sulfur vapors or gases. It mixes easily with oils and other colors.³ It is insoluble in water, ammonia and alcohol and is practically fire proof.⁴ Carbon dioxide and dilute acid vapors have no effect on it. Unless specially treated light causes it to darken but it becomes white again when it is removed from the light.

This pigment is made by the double decomposition of solutions of ZnSO_4 and BaS in equimolecular proportions so that the product consists primarily of 30% ZnS , 69% BaSO_4 , and 1% ZnO . When so precipitated, lithopone shows the characteristic properties which make it so desirable as a pigment. That it is not a mere mixture becomes evident when molecular proportions of dry ZnS and BaSO_4 are thoroughly ground together. Under these conditions the mixture does not exhibit the same properties as pure lithopone.

An attempt will therefore be made to explain the mechanism of the formation of lithopone which may in part account for its characteristic properties. According to Mukherjee⁵ and others "for most suspensoids the charge is of the same sign as that of the ion the substance has in common with the peptizing or stabilizing electrolyte." So metallic sulfides with a stabilizing electrolyte when the common ion is S'' or SH' will have a negative charge. Similarly barium carbonate in barium nitrate has the barium ion in common with the electrolyte and the barium carbonate becomes positively charged. Also it is a known fact that oppositely charged colloids mutually precipitate each other.⁶

When ZnSO_4 solution is added to a solution of BaS or $\text{Ba}(\text{SH})_2$ barium sulfate and zinc sulfide form. As the first particle of each of these materials forms, it is in contact with a large amount of electrolyte having the barium ion common with the BaSO_4 and the S'' or SH' ion common with the zinc sulfide. The barium ions are adsorbed over the

surface of the barium sulfate particle due to the same causes responsible for crystal growth.⁵ As there is not a similar number of SO_4 ions available in the electrolyte the barium sulfate particle does not grow but because of the attached positive ions on its surface it assumes the same charge as the barium ion and becomes positive. At the same time and in a similar way the ZnS attracts the negative S'' or SH' ion to the surface and becomes negatively charged. The two oppositely charged particles of BaSO_4 and ZnS immediately unite by the neutralization of the charges and form a product which has neither the properties of BaSO_4 or ZnS nor of a common mixture of the two. It is due to this union of the charged particles that the properties of lithopone may be ascribed.

To determine the validity of this assumption a positive barium sulfate colloid, and negative zinc sulfide colloid were prepared, these brought together and the precipitate which formed tested for the properties of lithopone.

The barium sulfate colloid was prepared by slowly adding a solution of sulfuric acid in 60% alcohol to a solution of barium chloride in 60% alcohol with constant stirring. A slight excess of barium chloride was used. A colloidal solution containing 0.1 gm. barium sulfate per cc. could thus be obtained which remained in suspension for twenty-four hours.

An apparatus proposed by H. Taylor⁷ which had been successfully used by H. B. Weiser⁸ to determine the charge of BaSO_4 was used. The apparatus consisted of a thin walled "U" tube two millimeters internal diameter. To the bottom of this "U" was connected a tube of similar diameter which had a constriction in it near the point of connection to the "U" tube through which the colloidal solutions were introduced. Small platinum wires in the form of spirals introduced three centimeters into the tubes were used as electrodes and a direct current of 110 volts applied. About three centimeters of distilled water covered the colloidal solution in each leg of the "U" tube. At the end of thirty minutes a very definite interface between the clear liquid and the turbid colloidal solution was noted at the positive electrode and the turbidity around the negative electrode increased. At the end of an hour the interface had lowered 3.75 cm. This clearly indicated that the BaSO_4 as prepared was positively charged.

A ZnS colloidal solution prepared according to a method given by Taylor⁹ by passing hydrogen sulphide through ammoniacal zinc hydroxide solution with a small amount of gum arabic added showed a like interface at the negative electrode indicating that the ZnS was negatively charged.

When solutions containing equimolecular quantities of these colloidal solutions were brought together a heavy precipitate formed, the pre-

precipitation being complete in twenty minutes. This precipitate when washed and dried appeared similar to commercial lithopones under the microscope. The particles were extremely finely divided and uniform in size and of amorphous character. A seven per cent hydrochloric acid solution produced no H_2S detectable with lead acetate paper.

To simulate the conditions under which the colloids would form in making lithopone, another method was used to prepare the colloids. A barium sulfate colloid was made by grinding barium sulfate in a barium sulfide solution of 4° Bé. in a ball mill for fifty hours. For this purpose 50 gms. of the barium sulfate were ground with 250 cc. of the sulfide solution which supplied the common barium ion. The practically clear solution obtained after filtering through a double thickness of filter paper when tested in the Taylor apparatus showed that a positively charged barium sulphate colloid had been obtained.

In like manner, zinc sulfide ground with a solution of barium sulfide in a ball mill produced a negatively charged zinc sulfide due to the presence of the common sulfide ion present in the solution.

When these solutions are brought together a precipitate forms, which when washed and dried produces a substance which has the properties of a true lithopone.

If this is the mechanism of lithopone formation there should be possible a method of producing lithopone by grinding together barium sulfate and zinc sulfide in the presence of barium sulfide solution. It should be possible also to substitute zinc sulfate for the barium sulfide inasmuch as the zinc sulfate electrolyte has the Zn ion common with the ZnS and the SO_4^{--} ion common with the barium sulfate. In this case the zinc sulfide would become positively charged and the barium sulfate negatively charged.

Experiments have been tried to make lithopone by this grinding method but the work has not progressed sufficiently to warrant any statement as to its possible success.

From these considerations it seems reasonable that in the mutual precipitation of the barium sulfate and zinc sulfide each takes on a charge which in the neutralization of one by the other forms a union of the colloids and this union may be responsible for the characteristic properties of lithopone.

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Division of Chemical Engineering,
University of Minnesota, Minneapolis, Minn.

AN EXPERIMENTAL STUDY OF EMULSIFICATION ON THE BASIS OF DISTRIBUTION OF SIZE OF PARTICLES

BY ALFRED J. STAMM

The realization in recent years of the great importance of emulsions and of emulsification has been a great incentive for investigators to try to build up a satisfactory theory explaining the phenomena. A large amount of experimental work has been done on the subject, but no satisfactory all-embracing theory of emulsification has resulted. There appear to be two general causes for this. In the first place, the emulsifying agents used in stabilizing emulsions differ greatly in character. Holmes and Williams¹ have shown that methyl alcohol will stabilize emulsions of benzene in water. In this case we are dealing with a simple, soluble, non-colloidal emulsifying agent possessing definite polarity. In the case of soaps we are dealing with less soluble colloidal emulsifying agents, definitely polar, and displaying evidences of complexities due to dissociation and association. In the case of protein emulsifying agents we are dealing with complex material of indefinite chemical identity both as to structure and number of molecular species present, showing colloidal behavior, but quite questionable as to the existence of any polarity. In the case of Pickering's² solid emulsifying agents we are dealing with insoluble particles varying from colloidal dimensions to those vastly beyond the colloidal range, which could not conceivably possess polarity in the same sense that soap possesses it. Due to these great differences in the properties of the materials which function as stabilizing agents it becomes difficult to explain their action upon any one property or set of forces as so many investigators have tried to do. If such a thing as a complete inclusive cause of emulsion stability could be evolved it would probably be made up of quite a few factors each of which would vary in importance in changing the type of emulsifying agent. It hence appears that the first difficulty in building up such a universal theory of emulsification is that investigators have confined their efforts to the study of emulsions using only one type of emulsifying agent. It is true that different investigators have studied different systems using different types of stabilizers, but on what basis can their results be compared? This question leads directly to the second diffi-

¹ Holmes and Williams, "Colloid Symposium Monograph," Vol. 2, p. 135.
² Pickering, *J. Chem. Soc.*, 91, 2001 (1907).

culty. A great deal of the experimental work on emulsions is purely of a qualitative nature. Further, the experimental data which are in general considered to be quantitative are data on the properties of emulsion constituents, and not on emulsions themselves. Such data, though very useful, give an inadequate characterization of emulsions. A true characterization of an emulsion might be made by determining some such property as its dispersity by a quantitative means. This can be done by determining the distribution of the size of the particles in the emulsion. With the aid of distribution curves it becomes possible to compare the data of different investigators, and data obtained from widely differing types of emulsions. It is also possible to tie up the quantitative properties of the emulsion constituents with a quantitative property of the emulsion itself.

The determination of the statistical distribution of the size of particles in suspension systems has been studied fairly extensively by Odén,³ and by Svedberg and his associates.⁴ Very few studies have been made, however, of the distribution of size of particles in emulsion systems with the objective of building up a satisfactory theory of emulsification. Finkle, Draper, and Hildebrand,⁵ and Harkins⁶ have determined the number distribution of size of particles in emulsions by microscopic measurement with this object in view. Grouping the particles into size classes, and plotting the number of particles in each class against the class size gave the number distribution curve directly. This method, though simple, is very tedious when enough particles are measured to justify the application of a statistical treatment. Further, such a number distribution curve does not adequately characterize the true dispersity of an emulsion. The additive mass of all of the particles including all sizes below that of the number maximum may amount to only a very small fraction of the total mass of dispersed material for many emulsions. A mass-distribution curve represents far more adequately the dispersity of an emulsion as it accounts for the total mass of dispersed material. Microscopic data can be converted to a mass basis by multiplying the number of particles corresponding to each class size by the mass of a particle of that size. This requires, however, the measurement of an exceedingly large number of particles to make the results statistically sound.

Two truly statistical methods of determining the distribution of the size of particles in emulsions have been developed, both of which are based on Stokes' law of settling. The first of these developed by Dr.

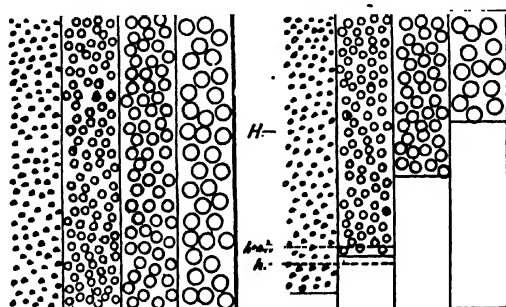
³ Odén, *Proc. Roy. Soc., Edinburgh*, **36**, 219 (1916).

⁴ Svedberg and Rinde, *J. Am. Chem. Soc.*, **45**, 843 (1923); Svedberg and Nichols, *J. Am. Chem. Soc.*, **46**, 2910 (1924); Svedberg and Rinde, *J. Am. Chem. Soc.*, **46**, 2877 (1924).

⁵ Finkle, Draper, and Hildebrand, *J. Am. Chem. Soc.*, **45**, 2780 (1923).

⁶ Harkins, *Science*, **59**, 408 (1924).

E. O. Kraemer and the author⁷ was described at the last Colloid Symposium. A description of the second method, developed by the author under the direction of Dr. The Svedberg,⁸ has just been published. These methods differ merely in the manner in which the rate of sedimentation is followed. Let us picture, for the sake of simplicity, a system containing but four size classes of emulsion particles less dense than the medium evenly dispersed throughout the medium. Fig. 1 shows these classes separated in a diagrammatic manner. Each of these classes of particles settles out under the influence of gravity at a definite rate dependent on its particle size, as described by Stokes' law. If the rate at which the total mass of particles passing a defi-



SEDIMENTATION SYSTEM

FIG. 1.

nite height is followed, it will be found to decrease as the various size classes pass completely above that height. The rate of decrease of this quantity in turn (the second derivative) depends directly upon the mass concentration of the size class that has just passed above the boundary. In this way the mass of particles corresponding to the different sizes is determined in the first method, by following the rate at which the particles pass the junction of a capillary manometer tube connected to the sedimentation tube. This is done by observing the rate of fall of the dispersion medium in the manometer, which is caused by the lighter dispersed particles rising in the sedimentation tube above the junction, and subsequent displacement of an equal volume of the heavier medium to a position below.

In the second method, the mass concentration of the dispersed particles is determined at different heights simultaneously. The difference

⁷ Kraemer and Stamm, *J. Am. Chem. Soc.*, **46**, 2709 (1924); Stamm, "Colloid Symposium Monograph," Vol. 2, p. 70.

⁸ Stamm and Svedberg, *J. Am. Chem. Soc.*, **47**, 1582 (1925).

in concentration at heights h and $h + dh$ is due directly to the mass concentration of that class size of particles that has passed height h but not height $h + dh$. The concentrations at the different heights are determined photographically by the intensity of light scattered at right angles to the source when the emulsion is placed in an illumination cell (Fig. 2). The emulsion cell C is placed inside an air cell A which is cemented into the thermostat T with an open slot Y at the front through which the cell C is photographed. On either side of the thermostat are two illumination boxes B, containing 100 watt Mazda lamps. With a

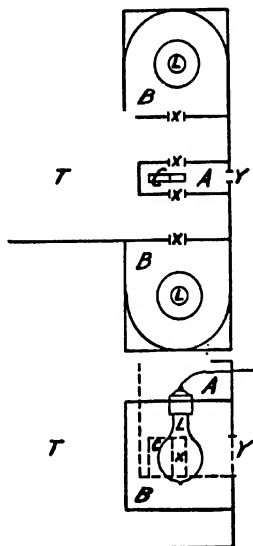


FIG. 2.—Apparatus for photographic method.

clear liquid in cell C, no light is scattered towards the front, but with a dispersed system in the cell the light scattered towards the front will depend on the number and size of the dispersed particles. The densities on the photographic plates are determined with a special photometer devised for the purpose.

The photographic measurements give the density-height relationship directly. This must be converted to a concentration-height relationship before the distribution of the size of particles can be determined. To make this transformation both the effects of the number of particles and the size of the particles on the intensity must be determined. With the use of two assumptions reasonable for dilute emulsions this relationship was determined theoretically and then verified experimentally. The

first of these assumptions is that when the concentrations of the systems are small, the manner in which one particle scatters light is independent of the manner in which adjacent particles scatter the light. From this it follows that the amount of light scattered towards the front of the cell depends directly on the number of particles scattering the light. Second, the particles are of such a size (diameters from 5-20 times the wave length of light) that the scattering may be considered to be primarily that of ordinary reflection. From this it follows that the amount of light scattered by a particle depends directly upon its surface, or the



FIG. 3.

Sedimentation Photograph

- (1) Immediately after placing in cell;
- (2) After 0.5 hours' sedimentation;
- (3) After 1.0 hours' sedimentation;
- (4) After 1.5 hours' sedimentation.

square of its radius. From these two assumptions the following relationship may be derived

$$\frac{dS}{dr} = kxI \frac{dD}{dx}$$

where $\frac{dS}{dr}$ is the distribution function, $\frac{dD}{dx}$ is the slope of the photographic density-height curve at height x , I the corresponding intensity of the scattered light, and k a constant of proportionality. Any element of area under a $\frac{dS}{dr}$ versus r curve represents the mass of the

particles dispersed between sizes represented by the bounding abscissa.^{8a}

Of the two methods the former is the most applicable for emulsions having concentrations of the dispersed phase greater than five per cent, and for emulsions that settle out relatively fast. The second method works best for emulsions of less than five per cent concentration, and for emulsions whose rate of settling is considerably slower.

The emulsions that have been studied in this investigation have all been of the type in which soaps serve as the emulsifying agents. For

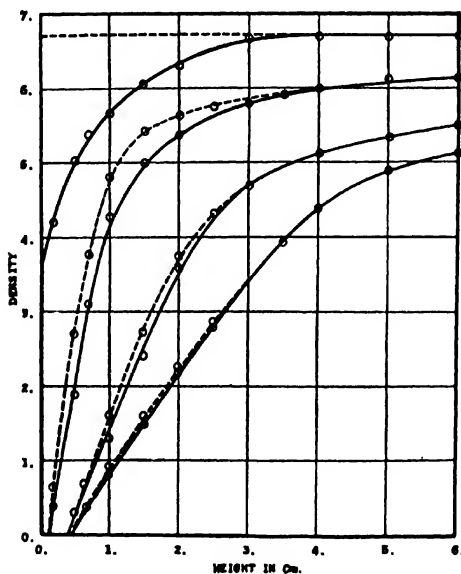


FIG. 4.—Density-Cell Height Curves corresponding to the above sedimentation periods

this reason, it will not be possible to draw any all embracing conclusions at the present time. The results, however, point to several interesting things concerning this type of emulsions. In the first place, the distribution curves indicate that the method of preparation affects the dispersity of benzene in water emulsions to a great extent. Homogenization with a Briggs' homogenizer under reduced pressure increases the dispersity of emulsions greatly. Curves obtained by the first method

^{8a} Fig. 8 gives a print of a photograph of a one per cent benzene emulsion 0.0005 N with potassium palmitate taken immediately after placing in the cell, and after 0.5, 1.0 and 1.5 hours sedimentation. Fig. 4 gives the corresponding density cell height curves.

given in Fig. 6 in the paper of Kraemer and Stamm⁷ illustrate this nicely. Further data obtained by the same method show that in a similar five per cent benzene in water emulsion 0.0005 N with potassium palmitate, that has been homogenized four times in a colloid mill, still more finely dispersed particles are obtained (Fig. 5, curve 2). The range of the particle size varies from ten-fold in the most perfectly homogenized emulsions studied to over fifty-fold, showing that uniform dispersity is far from being obtained.

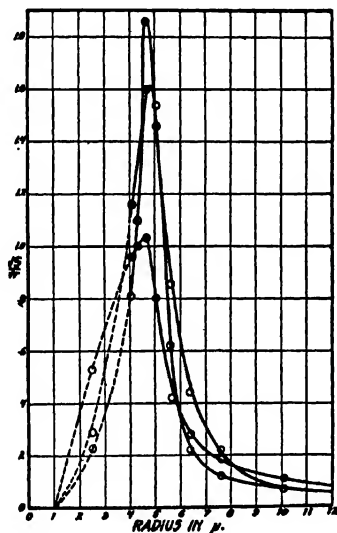


FIG. 5.

Distribution Curves, Method of Kraemer and Stamm
5% Benzene Emulsions 0.0005 N in.

- (1) Sodium palmitate (highest maximum)
- (2) Potassium palmitate (next highest maximum)
- (3) Cæsium palmitate (lowest maximum)

Figure 5 is of further interest. These three curves obtained by the first method represent the distribution of the size of the particles in five per cent benzene emulsions containing: (1) 0.0005 N sodium palmitate as stabilizers, that have been homogenized four times in a colloid mill. Similar determinations using the second method of determining the distribution of size of particles are given in Fig. 6 for one per cent benzene emulsions, 0.0005 N with soaps, and homogenized twice in a Briggs' homogenizer. It is evident that in the case of the mass distributions there is no appreciable shift in the maxima in

changing from one monovalent soap to another as is the case for the number maxima given by Harkins.⁹ Harkins found a decrease in the size of the particles corresponding to the number maxima in going from sodium to potassium to caesium soaps. As he had used more concentrated emulsions and had prepared them by a different means, it was found advisable to check his work using the concentrations and methods of preparation of this investigation. About 500 particles were measured per run. The sizes of the particles were somewhat less than

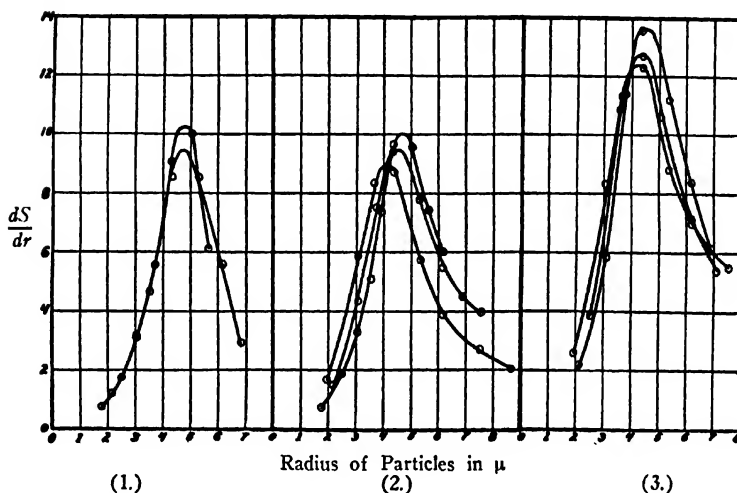


FIG. 6.—Distribution Curves, Photographic Method.
1% Benzene emulsions 0.0005 N in
(1) Caesium palmitate
(2) Potassium palmitate
(3) Sodium palmitate
Sedimentation periods varying from 0.5 to 1.5 hours.

those given by Harkins. The smallest class size that could be measured with the microscope used was of the order of 1.0 μ in diameter. No number maxima resulted above this size in any of the cases, but an increase in the number of the smallest particles in going from sodium to potassium to caesium was quite evident. If smaller particles could have been measured the results might very well have given similar results to those of Harkins. The data were converted to a mass basis and good checks obtained with the curves of Fig. 6 (Fig. 7). Further Harkins and Keith's¹⁰ own data gave on conversion to a mass basis

⁹ Harkins, "Colloidal Behavior," by Bogue, p. 202.

¹⁰ Keith, Thesis, University of Chicago (1924).

approximately constant mass maxima. According to this, the dispersity of an emulsion is chiefly due to the mechanical method of preparation rather than the specific nature of the emulsifying agent. It appears true that for the smaller particles certain specific forces of the emulsify-

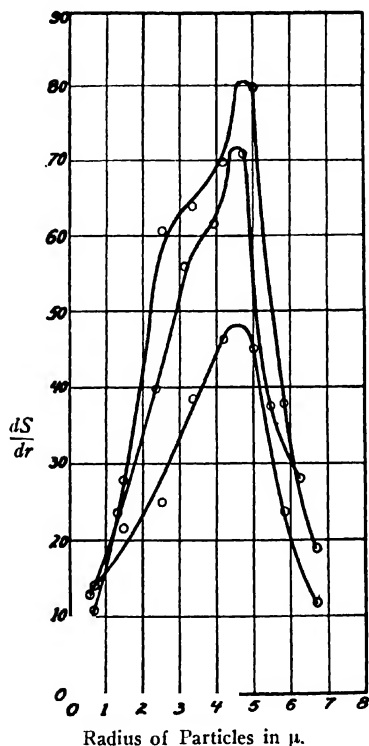


FIG. 7.—Distribution Curves, Microscopic Method.
5% Benzene emulsions 0.0005 N in
(1) Sodium palmitate (highest maximum)
(2) Potassium palmitate (next highest maximum)
(3) Cæsium palmitate (lowest maximum).

ing agent become appreciable in determining the size of the particles. It does not seem reasonable, however, to connect this force with any wedge shape that the oriented emulsifying agent molecule may possess, as Hildebrand⁶ and Harkins^{6, 9} have suggested. The assumption that such a packing effect of the oriented wedge-shaped molecules of soap

determine the curvature of the emulsion droplets would be justifiable if the molecules were actually packed, but this is not the case. Using N. K. Adam's¹¹ data for the cross section of the two ends of the palmitic acid molecule, it can be calculated that the diameter of the drops, where the packing of oriented acid molecules around the droplets is complete, would be $0.05\ \mu$. This is $1/20$ the size of the smallest drops detectable for potassium palmitate, which according to the theory should give still smaller drops. Even in the case of packing under pressure, as pictured by Adam,¹¹ the drops would have to be $1/10$ the size of the smallest drops, or $1/40$ the size of the drops corresponding to the surface maximum. This incompleteness of packing has also been observed by Harkins when the size of the particles corresponding to the number maximum is taken. When particles of the size at the surface or mass maxima are considered the packing is still less complete.

The presence of an excess of alkali is of importance in this connection. Harkins found that an excess of alkali decreased the size of the particles corresponding to the number maxima for all monovalent soaps. On repeating these measurements, in this laboratory using more dilute emulsions, no number maxima resulted. A definite increase in the number of smaller particles was obtained, however, as indicated in Table I. The results on a number basis on conversion to a mass basis give essentially constant maxima similar to the above case with different monovalent soaps.

TABLE I

EFFECT OF AN EXCESS OF POTASSIUM HYDROXIDE ON THE DISPERSITY OF 5 PER CENT BENZENE EMULSIONS STABILIZED WITH 0.0005 N POTASSIUM PALMITATE

KOH Conc.	Microscopic Measurements		
	Radius of Smallest Class	Per Cent of Particles in that Class	Radius at Mass Maximum
0.0	0.77-2.31 μ	23.3	9.1 μ
0.00085N	" "	49.5	9.0
0.00170N	" "	53.3	9.2
0.00085N	Sedimentation method, Kraemer and Stamm ¹		9.2

It is evident that the mechanical means of preparation again is the chief factor in determining the degree of dispersity of the emulsions, but there is also a tendency for an excess of alkali to decrease the size of the smaller particles. Harkins⁹ showed that this decrease in the size of the smaller particles is in agreement with the wedge theory of emulsification, as an excess of KOH represses the hydrolysis of potassium palmitate, and hence cuts down the proportion of oriented palmitic

¹¹ Adam and Dyer, *Proc. Roy. Soc. (London)*, 106, 694 (1924).

acid molecules in the interface, which give larger particles than the soap.

Qualitative tests were made on the effect of an excess of alkali on the dispersity of water in amyl alcohol emulsions stabilized with barium palmitate. Amyl alcohol was used instead of benzene due to the slight solubility of barium palmitate in benzene. Comparison of the rate of settling of the two emulsions, identical except for an excess of barium hydroxide in the one case, showed that the particles settled considerably faster when there was an excess of alkali. Microscopic examination after half an hour's settling showed that fewer and larger particles were dispersed in the alcohol with an excess of barium hydroxide. In this case the dispersity of the smaller particles of water in oil emulsion stabilized with a bivalent soap is decreased upon addition of the corresponding hydroxide. This is diametrically opposed to the deductions which follow from the wedge theory of emulsification. If the barium hydroxide cuts down the hydrolysis of barium palmitate, the particles should be still smaller. This is true because the palmitic acid normally present tends to give the reverse type of emulsions.

Further, data given by Seifriz¹² are similarly in opposition to the wedge theory. This investigator worked with petroleum oil-water emulsions stabilized with an aqueous colloidal suspension of casein. The light oil fractions gave oil in water emulsions, whereas the heavy oils gave the reverse type. Sodium hydroxide, barium hydroxide, and several salts were found to stabilize the oil in water emulsions, whereas the same bases and salts decreased the stability or even broke the emulsions of water in oil. The effect on the oil in water emulsions might be reconciled with the wedge theory if it were certain that casein is a definite salt which undergoes hydrolysis. The effect of barium hydroxide upon the water in oil emulsions, on the other hand, is diametrically opposed to the theory as before. Further, sodium hydroxide and barium hydroxide have the same effect, in the first case, of stabilizing, and in the second of breaking the emulsions.

The inadequacy of the wedge theory to account for the relatively large size of emulsion particles, and also the decrease in dispersity of water in oil emulsions upon addition of alkali has been pointed out. If the oriented molecules never pack so as to touch along their length, but merely tend to curve that way, why should the effect be considered due to the shape of the soap molecules rather than some specific force exerted between the oriented soap molecules in the interface? It has been shown by Finkle, Draper and Hildebrand⁵ that the phase relationship cannot always be determined by the generalization that the phase in which the emulsifying agent is soluble forms the external phase. These investigators cited as an exception the case of a free fatty acid

¹² Wm. Seifriz, *J. Phys. Chem.*, **29**, 587 (1925).

which is soluble in oil and not in water forming an oil in water emulsion. In this investigation, an interesting case was found where the emulsifying agent is soluble in both phases. Emulsions of amyl alcohol in water result regardless of which phase the soap is dissolved in, and from qualitative tests appear to give the same dispersity. The matter of solubility alone hence cannot account for the force determining the curvature of the interfacial film. The size of the particles appears to be related to the spreading coefficient of the dispersed phase on water for different systems as shown by the data of Harkins, that is, on the difference between the adhesional and cohesional work.⁹ Besides the mechanical forces of preparation, and this spreading coefficient,

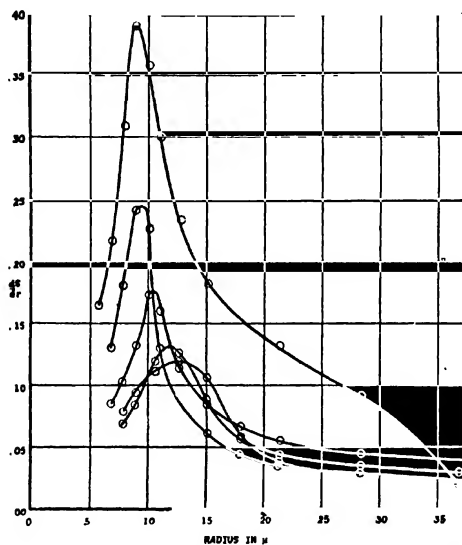


FIG. 8.—Distribution curves, method of Kracmer and Stamm, 5% benzene emulsions with potassium palmitate, palmitic acid, and mixtures of both as stabilizers.

there must also be some directing force exerted by the emulsifying agent itself. This tendency towards curving the oriented film might be explained on the basis of an electrical effect rather than the wedge shape of the molecules. In general, water tends to form the external phase of an emulsion. Emulsions of water with amyl alcohol, benzene, heptane, stanolax, dimethyl aniline, nitro-benzene, chloroform, and carbon tetrachloride, using both monovalent soaps and fatty acids as emulsifying agents, give oil in water emulsions. This same group of organic liquids give oil in glycerine emulsions with glycerine. The fact that water and glycerine both form the external phases might be due

to the fact that each has a considerably higher dielectric constant than the other liquids. Any repulsive force that might be exerted between the like ends of oriented soap molecules in the interface would be magnified in the liquid of high dielectric constant, and thus tend to cause a curvature in such a direction as to make it the external phase. In the case of the bivalent soaps, the number of carboxyl groups that can repel each other is cut in half due to their being held in pairs by the bivalent metal, whereas the number of hydrocarbon groups that may repel each other remains the same as before. This effect may be more than enough to overcome the difference in dielectric constant and thus give the opposite type of emulsion. An increase of alkali in either case presumably has the effect of increasing this repelling force in the phase in which

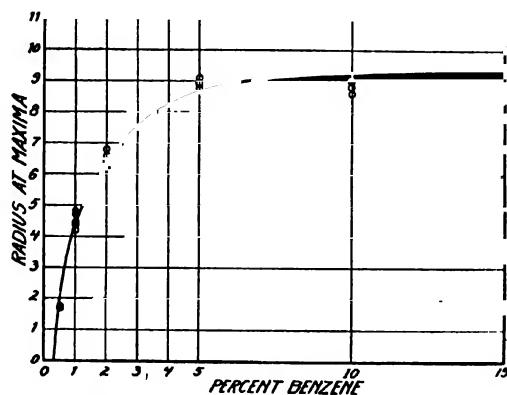


Fig. 9.—Change in radius corresponding to the mass maximum with changes in benzene concentration.

it is soluble, in this case the water, thus causing an increase in dispersity for oil in water emulsions, and a decrease in dispersity for the reverse type.

This notion is highly speculative and is offered merely as a possible suggestion in the absence of a sound theory. More experimental data of varying types are needed, especially that of an electrical nature to make any such theory sound.

The effect of palmitic acid as a stabilizing agent in emulsions of benzene in water was studied. The free fatty acid gave mass maxima corresponding to larger particles than those given by the soaps at the same total concentrations (Table II). When varying proportions of potassium palmitate and palmitic acid were used, intermediate mass maxima were obtained (Fig. 8, Table III). These data are in accordance with the wedge theory of emulsification, but do not necessarily

TABLE II

SIZE OF PARTICLES CORRESPONDING TO THE MASS MAXIMA FOR BENZENE EMULSIONS HOMOGENIZED TWICE IN A BRIGGS' HOMOGENIZER UNDER REDUCED PRESSURE

Soap	Conc. of Soap	Vol. Per Cent Benzene	Radius at Mass Maximum	Method	Remarks
Potassium palmitate	0.0005 N	15.0	10.2 μ	S *	Data from paper of Kraemer and Stamm
		10.0	8.8	"	
		10.0	9.0	"	
		10.0	8.6	"	
		5.0	8.9	"	
		5.0	8.8	"	
		5.0	9.1	M	1067 particles meas.
		2.0	6.3	P	
		2.0	6.5	"	0.5 hr. sedimentation
		2.0	6.2	M	0.75 " "
	0.0025 N	1.0	4.1	P	507 particles meas.
		1.0	4.5	"	0.5 hr. sedimentation
		1.0	4.7	"	1.0 " "
		1.0	4.7	"	1.5 " "
		1.0	4.5	M	456 particles meas.
		1.0	4.5	"	489 " "
		0.5	1.7	P	2.0 hr. sedimentation
		0.5	1.75	M	311 particles meas.
		5.0	6.5	"	750 " "
Sodium palmitate ..	0.0005 N	1.0	4.2	P	0.50 hr. sedimentation
		1.0	4.3	"	0.75 " "
		1.0	4.5	"	1.00 " "
		1.0	4.7	M	676 particles meas.
Cæsium palmitate ..	0.0005 N	5.0	8.8	"	307 " "
		1.0	4.7	P	1 hr. sedimentation
		1.0	4.8	"	1.5 " "
		1.0	4.5	M	1076 particles meas.
Palmitic acid	0.0005 N	5.0	12.1	S *	771 particles meas.
		5.0	12.5	"	
		5.0	12.0	M	
		1.0	6.9	P	
		1.0	6.6	"	0.267 hr. sedimentation
		1.0	6.6	"	0.50 " "
		1.0	6.6	"	0.75 " "
		1.0	6.7	"	0.267 " "
		1.0	6.5	"	0.517 " "
		1.0	6.5	"	0.75 " "

* S. Sedimentation method of Kraemer and Stamm.⁷

M. Microscopic method.

P. Photographic method.

substantiate it as the two emulsifying agents, soap and free fatty acid, enter the drop interfaces from the different phases in which their concentrations are not alike. The distribution ratio of palmitic acid between its true solution in benzene and the interface might be quite dif-

ferent from that between the colloidal aqueous soap solution and the interface. In the next section, the effective concentration differences will be shown to cause differences in the dispersity of the emulsions. The areas under the curves in Fig. 8 decrease when going from pure potassium palmitate as the emulsifying agent to palmitic acid. This is due to the fact that when the acid is used as a stabilizer, part of the benzene is so coarsely dispersed that it settles out immediately. It is interesting to note that at both one per cent and five per cent benzene concentrations, mixtures of the two emulsifying agents give intermediate mass maxima though the emulsions correspond to quite different portions of the curve of Fig. 9.

This curve (Fig. 9) shows the shift of the mass maxima for benzene emulsions of different concentration with constant soap content (0.0005 N in potassium palmitate). The mass maxima increase exponentially with an increase in benzene concentration. When both the benzene and soap concentrations are increased in the same proportion, a maximum corresponding to a larger size of particles is obtained (see Table II). This shows that the efficiency of homogenization decreases with an increase in total concentration. This effect, as well as that of the relative soap concentrations, is effective in determining the nature of this curve.

TABLE III
EFFECT OF AN EXCESS OF PALMITIC ACID ON THE MASS MAXIMA

Per Cent Benzene	Palmitic Acid	Potassium Palmitate	Radius at Maximum of Distribution Curve	Method
5.0	0.0005 N	0.0 N	12.5 μ	Kraemer and Stamm
"	.000375	.000125	11.7	
"	.00025	.00025	10.3	
"	.000125	.000375	9.4	
"	.0	.0005	8.9	Microscopic
"	.0005	.0	12.0	
"	.00025	.00025	10.0	
"	.0	.0005	9.1	
1.0	.0005	.0	6.6	Photographic
"	.00025	.00025	5.3	
"	.0	.0005	4.4	

The mass distribution curves from which Fig. 9 was obtained were transposed to a surface distribution basis by dividing each value of $\frac{dS}{dr}$ by the corresponding average value of r . This caused but a slight shift in the maxima to a position corresponding to slightly smaller par-

ticles. The total surface of the dispersed particles was approximately determined by taking the areas under these curves extrapolated to the axis of abscissæ. The values are given in Table IV. With these total surfaces, and the molecular cross section of the adsorbed soap molecules an approximation can be made as to the thickness of the adsorbed layer in terms of the number of molecules thick. The cross section of the palmitic acid molecule for the carboxyl or larger end as given by Adam¹² is 25.1×10^{-16} cm.² With the use of this cross section and Avogadro's number, the number of mols of soap required to cover the total surface one molecule thick can be calculated.

TABLE IV
BENZENE IN WATER EMULSIONS STABILIZED WITH 0.0005 N SOAPS

Soap	Per Cent. Benzene	Surface per 100 cc. of Emulsion in cm. ²	Mols Cover Surface	Molecules Thick for Complete Adsorption	Method of Prep.
Potassium palmitate	1.0	0.574×10^4	0.377×10^{-4}	13.2	Briggs' homogenizer
" "	2.0	0.995	0.653	7.65	
" "	5.0	1.35	0.886	5.65	
" "	10.0	2.17	1.42	3.52	
" "	15.0	3.27	2.15	2.33	
" "	5.0	2.75	1.81	2.76	Colloid mill
Cæsium palmitate...	5.0	2.65	1.73	2.89	
Sodium palmitate ..	5.0	2.75	1.81	2.76	

The ratio of the number of mols of soap present to the number required to form a monomolecular interfacial layer gives the number of molecules thick that the film would be if adsorption were complete. Complete adsorption can hardly be expected as there should be a distribution equilibrium between the interface and the solution. Briggs¹³ found that in a benzene water emulsion, containing sodium oleate in about the same concentration as the potassium palmitate in the above, that approximately one-fourth of the soap was adsorbed. It hence seems probable that the adsorption approaches, if it is not already, one molecule thick when the proportion of soap to benzene is small. Plotting the total surface (Table IV) of the dispersed benzene particles against the volume of benzene gives a straight line. This again shows that the dispersity of an emulsion is chiefly due to its mechanical treatment, for doubling the benzene concentration doubles the total surface of the dispersed particles as long as there is sufficient soap to form a monomolecular layer. Further, when the method of preparation is changed for similar

¹³ Briggs, *J. Phys. Chem.*, 19, 210 (1915).

emulsions, a considerable change in the total surface results. It is of interest to note also that the different soaps give not only the same mass maxima, but show the same total surface of the dispersed particles.

I wish to state my indebtedness to Dr. The Svedberg and Dr. E. O. Kraemer for making the development of the two methods of determining the distribution of the size of particles possible. I also wish to thank Prof. J. Howard Mathews for his interest and cooperation in this research.

Summary

1. The need for a quantitative means of determining the dispersity of emulsions is discussed.

2. Two statistical methods of doing this by determining the distribution of the size of particles have been developed, the first depending upon the determination of the rate of change of concentration with time at a fixed height in a sedimenting system, and the second depending upon the determination of the rate of change of concentration with height at a fixed time.

3. The first method is most advantageous for emulsions of over five per cent concentration that settle out rather rapidly. The second works best for more dilute emulsions that undergo a relatively slower rate of sedimentation.

4. The method of preparation of the emulsions is shown to be the prime factor in determining the dispersity.

5. Different monovalent soaps give the same mass maxima, and show the same total surface of dispersed particles.

6. An excess of alkali is shown to increase the dispersity of the oil in water type of emulsions, but to decrease the dispersity of the reverse type for the smaller dispersed particles, though it has no effect on the mass maxima in the first case.

7. The "Wedge Theory of Emulsification" is shown to be inadequate to explain the above results. A suggestion is made upon which a more feasible theory might be built.

8. Palmitic acid gives a mass maximum corresponding to larger particles than that given by the soaps. Mixtures of palmitic acid and potassium palmitate give intermediate maxima.

9. An increase in benzene concentration with constant soap content causes a shift in an exponential fashion of the mass maxima.

10. Calculations using the total surface of the dispersed particles show that the interfacial films of soaps are or approach monomolecular dimensions when the proportion of soap to dispersed material is small.

Laboratory of Colloid Chemistry,
University of Wisconsin, Madison, Wis.

THE CENTRIFUGAL METHOD FOR THE DETERMINATION OF THE DISTRIBUTION OF SIZE OF PARTICLE OF SUSPENDED MATERIAL

BY J. B. NICHOLS,* AND MISS HENRIETTE C. LIEBE.†

An accurate knowledge of the percentage distribution of size of particle of colloidal material is rapidly becoming one of the fundamental requirements for the control and improvement of product in the paint, dyestuff, rubber and ceramic industries.

There are a number of general methods in use at present. The photomicrographic method of Green¹ gives consistent results for particles larger than $.3\ \mu$ in diameter, provided that a sufficiently large number of particles are taken to apply a statistical method. The resulting distribution curve is on a number basis, however, so the maximum frequently occurs where there is only a very small percentage of the total weight of material. Such a curve may of course be transposed to a weight distribution curve by suitable means but the curve obtained is then only approximate.

The weight distribution curve may be obtained directly by the observation of the rate of settling or the rate of accumulation of the material suspended in a convenient medium. In the case of gravity sedimentation the particles are settling at a constant velocity, so the ordinary form of Stokes' Law may be applied to determine the size. Odén's² method of sedimentation onto an automatically recording balance is very convenient for particles not too small. Also the method of observing the apparent rate of change of density at a given point in a sedimenting system gives good results. The measurement of the change of concentration of the sol with height³ after a given time has elapsed is another very promising method.

Sedimentation under gravity is a time consuming process so if we substitute centrifugal force, we may determine the distribution relation most conveniently. This method depends on the determination of the change in concentration of the material with distance out from the axis of rotation after a given time of centrifuging. In order to accomplish

* University of Wisconsin.

¹ *J. Franklin Inst.*, 192, 687 (1921).

³ Svedberg and Rinde, *J. A. C. S.*, 45, 948 (1923).

† Acme White Lead-Color Works.

² Odén, *Kol. Zeit.*, 17, 88 (1916).

this, the rotor of the centrifuge must be designed so as to permit the observation or photographing of the material as it is being whirled out to the periphery. Such a centrifuge was designed at the University of Wisconsin during Professor Svedberg's visit ⁴ and later materially improved in Upsala.⁵

Figure 1 represents the centrifuge devised. The rotor A is directly connected at B to a Dumore special 20,000 r. p. m. motor C suspended in the heavy metal casing D and driven by a battery of storage cells to insure constant speed. The rotor is inclosed in a metal box G for the purpose of protecting the tube from air currents and also of decreas-

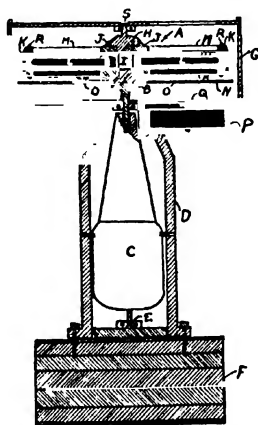


FIG. 1.—Centrifugal apparatus.

ing the air resistance. That the air resistance is quite appreciable is well shown by the fact that a speed of 2,500 r. p. m. with the box totally inclosed sinks to about 1,900 r. p. m. with the cover removed.

The rotor consists of the central head H, horizontally cored I, to which are screwed the two arms J, also cored to correspond to the core of the head. These arms are closed at the outer end by screw-caps K to provide a means for changing the tubes L contained. In order to obtain vertical or horizontal illumination of the tubes, the arms are slotted M, top and bottom and on both sides. The tubes used I, one for each arm, are made of Pyrex glass, and are closed at the inner end by paraffined corks.

A thin metal disc N, of slightly greater diameter than the length of the rotor, is attached to the head just below the arms. This screen is

⁴ Svedberg and Nichols, *J. A. C. S.*, 45, 2910 (1923).

⁵ Svedberg and Rinde, *J. A. C. S.*, 46, 2677 (1924).

slotted at O directly under the vertical slots in the arms and is fixed in position so that no relative motion of arms and disc can take place. This slotted disc therefore allows light to travel up through the box only when an arm is directly over the narrow beam of light employed for illumination. One of the slots is covered with black paper so that only one arm is actually observed.

Underneath the box is mounted a matte surface P for directing a uniformly diffused beam of light up vertically through a slot Q in the bottom of the box. This slot is of the same length as the slots in the arms so that every time an arm passes over this slot a beam of light travels up through the slots in the arm and through a corresponding slot in the top of the box, where the light not absorbed by the contents of the tube may be observed or photographed.

One of the most important requirements that a sedimentation centrifuge must meet is freedom of vibration, or in other words, perfect balance. After much experimentation a simple method of balancing was developed, depending on the fact that if the unbalanced motor is mounted flexibly, at a certain critical speed, the amplitude of vibration is a maximum. The motor and rotor are removed from the casing and supported in a horizontal position. The base of the motor is fixed but the end near the rotor is mounted on a very flexible thin steel strip. When the motor is started up, the unbalance produces a maximum amplitude of vibration vertically of the rotor at about 500 r. p. m. If the top of the rotating shaft is chalked at the maximum amplitude, the start of the chalk mark on the shaft coincides with the point of unbalance on the rotor. The shaft must be chalked at just the right time while the speed of the motor is passing through the critical range, for marks made below the critical speed frequently vary more than 100° from those made much above the speed of maximum vibration. With a little practice, however, the observer will chalk the shaft at the right time and can estimate the amount of unbalance and correct it. When the rotor has once been put in balance, the only two points of unbalance henceforth will be the arms. Therefore, when the chalk mark indicates the heavier arm, an adjustable ring threaded onto the end of the arm may be turned in till there is no vibration of the rotor at the critical speed.

The optical train consisted of a concentrated filament 400 W projection bulb, condensing lens, twelve inch water cell for cooling the beam of light, and a second lens at the focus of the first to throw a very uniform circle of light on the matte surface *p*. The diffused beam from *p* then travels up into the centrifuge through a slot in the bottom of the box. Now, when an arm passes over this slot, the light can continue up through the slots in the disc screen and the arm, eventually reaching the photographic plate through a 4.5 Tessar 1c lens, focal length 30 cm.

The intensity of the light is kept constant during an exposure by the adjustment of a small rheostat to keep the current flowing at 4.0 amperes. Time of exposure was usually controlled by use of a slide under the bottom slot since the general exposure time was one minute. For short exposures, a sector wheel could be used.

Speed regulation of the motor is provided for by inserting a variable resistance into the field circuit of the motor.

Theory of the Centrifugal Sedimentation of Uniform Sols

With the centrifuge the acceleration of the particle is no longer constant as in the case of gravity sedimentation but varies with the distance from the center.

Let a be the distance from the axis of rotation to the meniscus of the sol in the centrifuge tube. Then let x be the distance the boundary of the particles has moved in a given time t . Now consider the forces acting on a particle at the point x . The frictional force which tends to cause it to resist movement is $6\pi\eta r(dx/dt)$ where η is the viscosity of the liquid, r the radius of the particle considered to be a sphere, and dx/dt its velocity. But the centrifugal force applied to cause movement is $\frac{4}{3}\pi r^3 \cdot (d_p - d_l) \cdot \omega^2(x + a)$, $(d_p - d_l)$ being the difference in density between the particles and dispersion medium, ω the angular velocity, and $(x + a)$ the distance from the axis of rotation to the particle.

Equating and rearranging for integration

$$\int_0^t r^3 dt = \int_0^x \frac{9\eta}{2(d_p - d_l)\omega^2} \frac{dx}{x + a}$$

$$r = \sqrt{\frac{(9\eta \ln) \frac{x + a}{a}}{2(d_p - d_l)\omega^2 t}} \quad (1)$$

Therefore, by measuring the distance x which the boundary of the sol has moved out in a time t , and obtaining the speed of the centrifuge it is possible to determine r .

One of the first questions to be answered in developing a distribution relation is: Does the concentration of a uniform sol remain constant with time of centrifuging? If the centrifuging does change the relative concentrations of the different sizes of particle we would be determining only the distribution of size after a certain time of centrifuging rather than the distribution in the original sol. As a matter of fact the concentration does decrease with time but it fortunately remains uniform with distance out from the axis of rotation.

Two factors produce this decrease in concentration: the centrifugal force increasing with distance out from the axis of rotation, producing a stretching of a given element, and the tendency of the particles to move out radially, producing a spreading of a given element. The correction to be applied for each factor depends simply on the ratio of the distance of the particle from the axis of rotation at the start to the distance at time t . This is derived as follows: ⁶

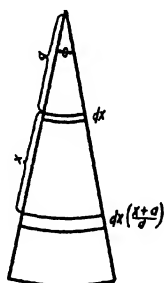


FIG. 2.—Annulus of Sector.

If we consider an annulus dx of a sector of angle θ and thickness p we find that the particles originally contained in dx will be stretched out within the annulus $dx \left(\frac{x+a}{a} \right)$ at time t . If n be the number of particles and dc and dc_t be the concentration within the two annuli, then

$$dc = \frac{n}{a \frac{\theta}{180} p dx}, dc_t = \frac{n}{(a+x) \frac{\theta}{180} p \left(\frac{a+x}{a} \right) dx}$$

$$\text{Combining, } dc = dc_t \left(\frac{x+a}{a} \right)^2 \quad (2)$$

The concentration of a uniform size of particle must therefore be multiplied by the factor $\left(\frac{x+a}{a} \right)^2$ to bring it back to the original concentration.

That the concentration does remain uniform throughout may be shown as follows: For a given size of particle the modified Stokes' formula may be transformed thus,

$$\ln \left(\frac{x+a}{a} \right) = \frac{2(d_p - d_1) \omega^2 t r^2}{9\eta}$$

⁶ Svedberg and Rinde, *J. A. C. S.*, **46**, 2684 (1924).

This becomes for a given time, speed, and size of particle

$$\ln \left(\frac{x+a}{a} \right) = C$$

or

$$\frac{x+a}{a} = e^r = \text{const.} \quad (3)$$

Now, if a varies, x must correspondingly change to retain the constancy of the relation. Substituting back in (2) we obtain

$$dc = dc_t e^{2r} \quad (4)$$

This shows that for a uniform size of particle the concentration decreases with time at a constant rate independent of the distance from the axis of rotation.

Since, in the special case of uniform sols $dc = c$, the equation $dc = dc_t \left(\frac{a+x}{a} \right)^2$ can be given the form

$$r = \frac{9}{2} \frac{\eta \ln \sqrt{\frac{c}{c_t}}}{(d_p - d_l) \omega^2 t} \quad (5)$$

the size of particle of a uniform sol may be determined by measuring the decrease in concentration with time of centrifuging.

Non-Uniform Sols

Since most colloidal material contains a wide range of size of particle the method just described for observing the movement of the boundary can give us complete information on only a few substances or at most the smallest size of particles contained in a non-uniform sol. Therefore the next problem is to work out the method for determining the relative amounts present of each size of particle in a sol. In a thin layer dx of a sedimenting sol the change in concentration dc from the section at x to the section $x+dx$ is due to particles of radius r to $r+dr$, the values of r being determined from the modified form of Stokes' law, Equation (1). By obtaining the change in c with x ,

that is dc/dx , we may determine the distribution function $\frac{dc}{dr} = \frac{dc}{dx} \cdot \frac{dx}{dr}$.

In order to measure dx/dr let us rearrange Equation 1 to the form

$$\ln \frac{x+a}{a} = \frac{2(d_p - d_l) \omega^2 r^2}{9\eta}$$

$$\text{Let } B = \sqrt{\frac{9\eta}{2(d_p - d_l) \omega^2 t}}$$

Substituting and differentiating,

$$\frac{dx}{x+a} = \frac{2rdr}{B^2}$$

Substituting the value of r from Equation 1 and rearranging

$$\frac{dx}{dr} = \frac{2(x+a) \sqrt{\ln \left(\frac{x+a}{a} \right)}}{B}$$

a being again the distance from the axis of rotation to the point $x = 0$. This then gives

$$\frac{dc}{dr} = \frac{2(x+a) \sqrt{\ln \left(\frac{x+a}{a} \right)}}{B} \frac{dc}{dx}$$

But this gives only the distribution of size of particle after the sol has been centrifuged a time t , so to obtain the distribution in the original sol, Equation (2) must be employed, giving

$$\frac{dc}{dr} = \frac{dc}{dx} \cdot \frac{2(x+a)^3 \sqrt{\ln \left(\frac{x+a}{a} \right)}}{a^2 B} \quad (7)$$

Method of Operation

The two centrifuge tubes were filled to the same height with the material to be studied inserted into the arms of the rotor, one of which is made opaque to the beam of light, and the rotor balanced up on the device previously mentioned. Before a determination is made the motor should be run at least two hours so that the rate of exchange of heat with the surroundings is constant. The temperature of the rotor is then from 3° to 4° higher than the room temperature so the tubes should be warmed up correspondingly before they are inserted into the arms.

Immediately after the sedimentation run was started a picture was taken of the contents of the tube to give a check on the uniformity of the original distribution, uniformity of light, and the density corresponding to the original concentration. Then pictures were taken at given intervals of time to give checks on the distribution curve. These were all taken on the same plate for the plate holder was arranged to allow five independent exposures on the same plate. At the conclusion of a run the speed of the motor was taken with a speed counter, the motor stopped, and the temperature of the contents of the tube recorded.

On the same plate an exposure was made of the pure dispersion medium under the same conditions as the other exposures in order to give the value of the photographic density corresponding to no material in suspension.

All plates were developed 10 minutes in ferrous oxalate developer, to which had been added 1 cc. 10% KBr solution per 100 cc. developer to repress fog. It is important to use the same amount of KBr each time for it not only prevents fog but depresses the characteristic curve of the plate proportionally to the amount added. For this latter reason the plates should also be tilted back and forth constantly during the development to cause uniform diffusion of the soluble bromides out of the plate. The plate should not be wet before putting it into the developer either for this would wash out some of the soluble bromide incorporated into the emulsion and thereby give a corresponding increase in density on development.

The next step was the photometering of the exposures. A modified König-Martens spectrophotometer was used. This was equipped with two brass carriages operated by micrometer screws and carrying an Eastman Kodak neutral wedge, density 4.0, and compensating wedge so mounted that the wedges could be moved up and down in front of the photometer slits. The exposure to be photometered was clipped in place on the compensating wedge and, with the eyepiece nicol at 45°, equality of transmissions was obtained by moving the neutral wedge up or down until the two fields were of the same brightness. The green mercury line (5461 Å) was used for the photometering.

Densities of the strip to be photometered were thus obtained in terms of the neutral wedge and could be converted to true densities by multiplying by the slope of the density—neutral wedge distance calibration curve. The calibration of the neutral wedge was also checked by determining the densities of two photographic strips separately and then superimposing them to determine whether the combined density was the same as the sum of the separate densities.⁹

Conversion of Photographic Densities to Concentrations

By Beer's Law, which holds for colloids, the relation between the original intensity of the light and I_o , that transmitted by the sol and incident on the photographic plate is

$$I_o = I_e - K'_{po} \quad (8)$$

where I is the original intensity, K' the absorption constant of the colloid, p the thickness of the cell and c the concentration.

⁹ Stamm and Svedberg, *J. A. C. S.*, **47**, 1586 (1925).

Since the light transmitted by the pure dispersion medium is $I_p = I_0 e^{-K'c}$, equation (8) may be transformed to $I_0 = I_p e^{K'c}$ or

$$\ln \frac{I_0}{I_p} = p(K - K'c) \quad (9)$$

which gives us a relation between the light incident on the photographic plate for pure dispersion medium and for a given concentration of colloid.

But since $D :: \gamma \log I$ on the straight part of the photographic curve, γ being the contrast, we may substitute in (9):

$$D_0 - D_p :: \gamma_p \log_{10} e (K - K'c) \quad (10)$$

Here D_p is the density corresponding to the dispersion medium and D_0 the density corresponding to a concentration c of the colloid. Therefore

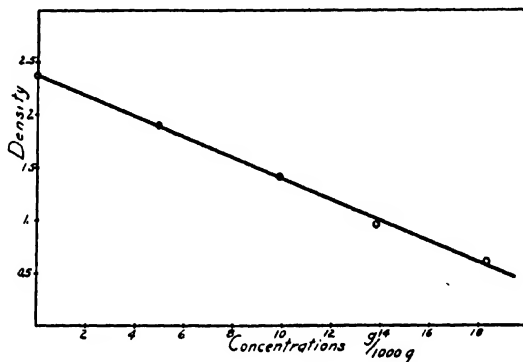


FIG. 3.—Density-concentration relationship before sedimentation.

the density-concentration relation should be linear. This was experimentally proven to be correct by making exposures of several different dilutions of the colloid in the centrifuge before appreciable sedimentation had taken place. The result is shown in Figure 3.

As the proportionality between density and concentration depends on factors that often vary appreciably in different plates, such as contrast, thickness of emulsion, and slightly different conditions of development, it is better to express the distribution function in terms of density rather than concentration directly.

If the slot in the rotor is not sector-shaped there will be a decrease in exposure from the inner end of the tube to the outer. As the slots of the present centrifuge are parallel sided, an exposure made of a uniform concentration will have a certain definite slope varying somewhat with different plates depending on the factors influencing the contrast

of the plate. Letting the point of highest density a represent the true concentration, an additive correction is applied equal to the product of the slope of the photometered density distance line multiplied by the distance of the point from a . This then gives a horizontal density line for a sol of uniform concentration before sedimentation has started, Figure 4a.

The same procedure is carried out for a photographic sedimentation curve Figure 4b. The lower part of the curve is quite evidently a straight line, an experimental proof of the previous considerations leading to the statement that beyond the boundary of the largest size class

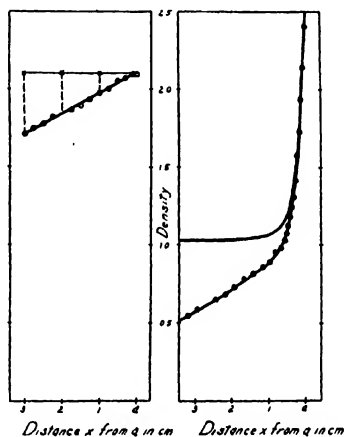


FIG. 4a.

FIG. 4b.

of particle, the concentration should be uniform in spite of the non-uniform field of force and the tendency to move out radially.

The slope of this lower part gives the correction to be applied to the curve to cancel the effect of decreasing time of exposure from the inner to the outer end of the tube.

After this correction has been made, the distribution curve is calculated in the usual manner by taking the tangents of this density distance

curve, yielding values of $\frac{dD}{dx}$. These are then multiplied by $\left(\frac{x+a}{a}\right)^2$

to give the change of density with distance in the original sol, and then by the corresponding values obtained from the modified Stokes' Law to give the change in density with radius of particle $\frac{dD}{dr}$. Plotting these

values against the corresponding values of r gives us the distribution curve.

Table I gives the data and computed quantities necessary in the determination of a distribution curve.

TABLE I

Speed 2340 r.p.m. ($\omega = 78\pi$). $T = 28.5^\circ$ ($\eta = 2.61$). $t = 90$ min.

$$a = 3.50 \text{ (3.28 reduced)}. \quad d_p = 4.3. \quad d_l = 1.25. \quad r = \frac{9\eta \ln \left(\frac{x+a}{a} \right)}{2(d_p - d_l)\omega^2 t}.$$

$$\frac{dx}{dr} = 1.683 \times 10^4 (x+a)r.$$

Distance x from Meniscus of Sol.	Radius r of Particle in μ	$\frac{dx}{dr}$	Density Correction for Exposure Variation $.55 \times$	$\frac{dD}{dx}$	$\frac{dD}{dx} \left(\frac{x+a}{a} \right)^*$	$\frac{dD}{dr}$	$.699 \frac{dD}{dr}$
.05 cm.	134 μ	$.751 \times 10^4$.026	16.39	16.8	12.63×10^4	8.84×10^4
.10	189	1.075	.052	9.91	10.53	11.31	7.92
.15	230.5	1.331	.078	8.235	9.01	11.97	8.38
.20	265	1.553	.104	7.675	8.66	13.43	9.40
.25	295.5	1.755	.130	7.120	8.27	14.70	10.13
.30	322	1.939	.156	6.152	7.355	14.26	9.98
.35	347	2.120	.182	5.094	6.245	13.22	9.26
.40	370	2.291	.208	4.428	5.588	12.80	8.96
.45	390.5	2.450	.234	3.813	4.942	12.09	8.46
.50	410.5	2.611	.260	3.250	4.325	11.29	7.90
.55	429	2.763	.286	2.838	3.874	10.70	7.49
.60	447	2.919	.312	2.476	3.455	10.09	7.06
.70	479.5	3.211	.364	1.888	2.778	8.99	6.24
.80	509	3.496	.416	1.531	2.369	8.27	5.79
.90	537	3.777	.468	1.342	2.179	8.23	5.76
1.0	562.5	4.053	.520	1.171	1.995	8.01	5.66
1.1	586	4.320	.572	1.004	1.794	7.74	5.42
1.2	609	4.593	.624	.818	1.529	7.01	4.91
1.4	650	5.120	.728	.451	.9112	4.66	3.26
1.6	687.5	5.648	.832	.1655	.3670	2.03	1.42
1.8	721	6.164	.936	.0655	.1575	.97	.68
2.0	752	6.683	1.04	0	0	0	0

Area = 143.0; reduced to 100 = $.699 \times 143.0$

Experimental

A series of six representative lithopones from different manufacturers form the basis for the experimental part of this paper. It was hoped that a study of the distribution curves would throw some light on the difference between the obscuring or hiding powers of the various lithopones as determined by Miss Liebe.

For the determinations, a .03 g. sample was rubbed up with 95%

glycerine in a mortar, adding a few cubic centimeters at a time till 100 cc. had been added.

Pictures with one minute exposure to the light transmitted by the sedimenting system were taken after 60, 90 and 120 minutes of centrifuging at a speed of 2,400 r. p. m.

The distance from the axis of rotation was taken as 3.5 cm. through-out. As the image of the contents of the tube is .937 actual size, a on the photographic plate is then 3.28 cm.

Viscosities of the 95% glycerine from 25° to 30° were determined and were considered to represent the viscosities of the sol.

Several check runs were made in most cases. Sometimes mixing occurred due to improper balancing. This not only tends to stir up the particles but seems to cause the concentration to decrease too rapidly with time, probably because particles that reach the proximity of the outer end on account of mixing, move out faster yet under the influence of the increased centrifugal force. As soon as they reach any part of the wall they stick fast.

Obscuring Powers

The obscuring power method for the determination of the fineness of pigments is in principle a turbidimetric method. The results obtained depend on the size of particle, difference in refractive indices of the disperse phase and medium, light absorption and reflection. The method is not applicable to material smaller than $.175 \mu$ in diameter but most pigments are above this limit.¹⁰ Experimentally, it has been determined that the depth of suspension just necessary to obscure an incandescent filament is inversely proportional to the concentration of the pigment.

The apparatus consisted of a Nessler tube graduated to 2 mm., set in a black box, provided with an aperture at the bottom to permit an electric light filament to send light up through the contents of the tube. For a determination, 0.25 grams of the lithopone were rubbed up in a porcelain mortar with seven drops of glycerine. The paste was thoroughly mixed for 3 to 5 minutes when 5 cc. of a 10% gum arabic solution were added dropwise, and with continued mixing of the suspension. Grinding action was avoided, inasmuch as obscuring power increases with increased grinding to a certain maximum. The suspension was then transferred to a 500 cc. volumetric flask and 100 cc. water added. After shaking the contents thoroughly, they were diluted to 500 cc. When necessary, a few drops of ether were added to remove the foam. The suspension was well mixed and a portion poured into a burette from which it was gradually run into a Nessler tube to a height just sufficient to completely blur the incandescent filament. At this point light which

¹⁰ Spear and Endres, *J. Ind. Eng. Chem.*, 15, 725 (1923).

passes through the column of liquid produces a "ground-glass" effect. Results are reported as obscuring power in square centimeters per gram of material.

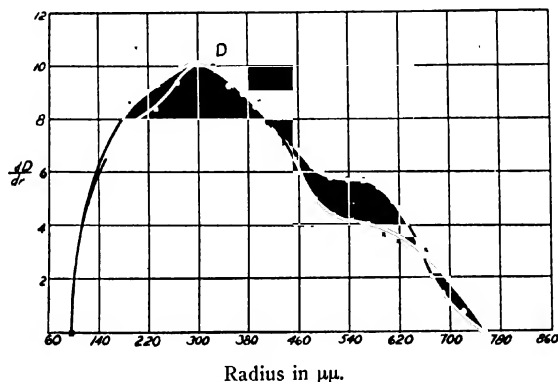
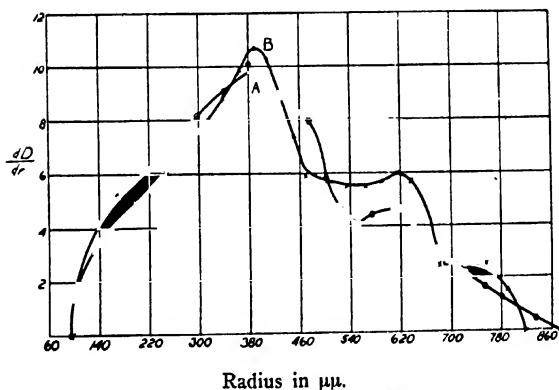


FIG. 5.—Distribution curves of lithopone D.



A. From exposure 60 min. after start.
B. From exposure 90 min. after start.

FIG. 6.—Distribution curves of lithopones.

Results

Figure 5 represents the distribution curve for lithopone D. This shows the average reproducibility of the curves. The scale has been so adjusted that each square equals four per cent of the total mass concentration of the material. While the form of the curve is not quite the same, the maximum at 300 $\mu\mu$, the indication of a maximum at 540 $\mu\mu$, and the limits are in good agreement.

Figure 6 shows the curves obtained for lithopone C for two exposures on the same plate representing 60 minutes' and 90 minutes' duration of centrifuging. Here also the two maxima 390 $\mu\mu$ and 620 $\mu\mu$ and

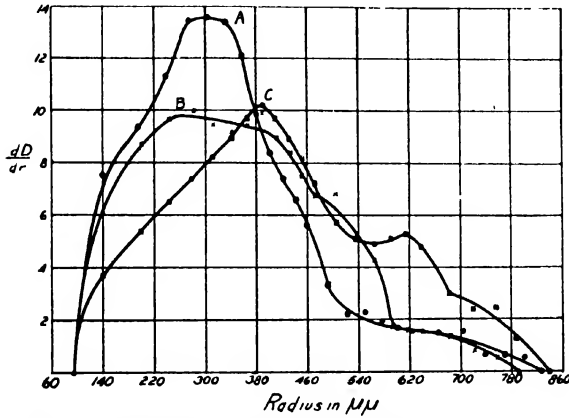


FIG. 7.—Distribution curves of lithopones A, B and C.

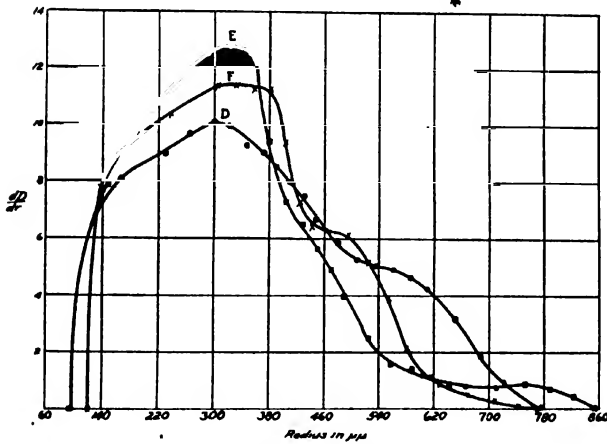


FIG. 8.—Distribution curves of lithopones D, E, and F.

the limits agree reasonably well. It is possible that slight coagulation has taken place between the two exposures, causing a shifting of material to the right.

Figures 7 and 8 show the distribution curves for the six lithopones. It will be noticed that the limits are nearly the same for all of them but

the maxima vary between 280 $\mu\mu$ and 390 $\mu\mu$. In order to compare these mass distribution curves with Green's¹¹ the area was divided up into radius intervals and the per cent of material in each divided by the cube of the average radius of the radius interval to give figures proportional to numbers of particles. Figure 9 shows the curves for *B*, *C*, and *E*. The maxima are at about 150 $\mu\mu$ (300 $\mu\mu$ diameter) checking roughly Green's values of .3 μ -.4 μ average diameter.

Based on the relative obscuring powers the rating should be: *B* best, then *E*, *A*, *D*, *F* and *C* in order. Comparing areas it is evident

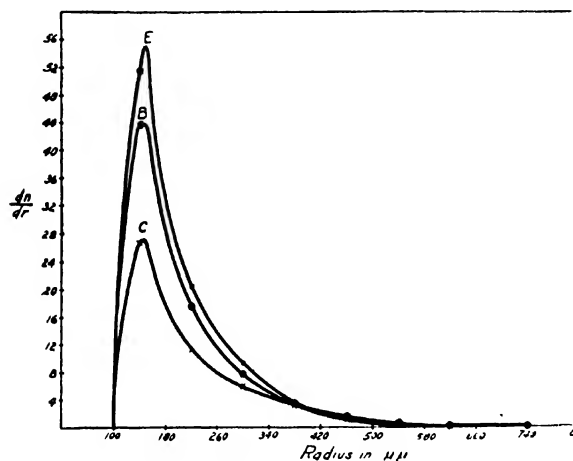


FIG. 9.—Number distribution of lithopones *B*, *C*, and *E*.

that *B* should have a higher obscuring power than *C*, since the smaller particles are most effective, but it is not at all evident that *A*, *E*, and *F*, having a greater proportion of smaller particles than *B*, should have a lower obscuring power than *B*. For the purposes of comparison the percentages obtained from the area under the curves have been tabulated for different radius intervals (Table II), together with the obscuring powers. To bring out apparent discrepancies we might compare *B* with *C* and *E*.

Lithopone	Per Cent in Range 100 $\mu\mu$ -260 $\mu\mu$	Obscuring Power cm. ² /g.
<i>B</i>	30.3	1501
<i>C</i>	19.2	833
<i>E</i>	35.4	1428

¹¹ *J. Franklin Inst.*, 192, 637 (1921).

TABLE II
PERCENTAGE DISTRIBUTIONS (FROM CURVES)

Radius Interval	Lith. A Per Cent	Lith. B Per Cent	Lith. C Per Cent	Lith. D Per Cent	Lith. E Per Cent	Lith. F Per Cent
100-180 $\mu\mu$	12.6	11.8	7.2	13.5	13.9	11.6
180-260	20.5	18.5	12.0	17.9	21.5	20.3
260-340	25.8	19.6	16.2	20.0	25.2	22.5
340-420	19.5	18.7	19.7	17.4	19.5	21.0
420-500	10.4	14.9	15.3	12.9	10.3	13.1
500-580	4.3	10.4	10.6	10.1	4.3	8.8
580-660	3.2	3.5	9.9	4.9	2.1	2.2
660-740	2.5	2.3	6.1	3.2	1.6	.5
740-820	1.2	.6	3.0	.2	1.6	...

OBSCURING POWERS $\text{cm.}^2/\text{g.}$

1309

1501

833

1164

1428

1072

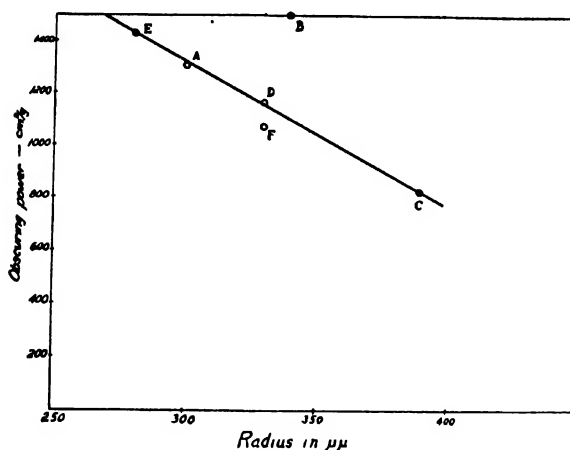


FIG. 10.—Relation of obscuring power to radius.

The soundest basis of comparison was finally found to be with respect to the weight mean radius, that is, that radius that divides the mass distribution into equal weights of material. This then corresponds in the mass distribution relation to the average radius of the number distribution relation.

Figure 10 shows the relation of the mass mean radius to obscuring power. The points corresponding to *B* and *F* at first seemed to invalidate the results but the following considerations offer a reasonable explanation. Since the zinc sulfide is on the outside of the BaSO_4 — ZnS

particles any oxidation of the lithopone during calcination will take place at the surface of the particles, forming zinc sulfate below 720°C . and zinc oxide above that temperature. The index of refraction of ZnS is 2.2-2.4, however, while that of ZnO is 1.9, ZnCO_3 , 1.16, ZnSO_4 , 1.46 and BaSO_4 , 1.6. Therefore any variation in the composition of the surface of the particles from ZnS , whether by oxidation, absorption of carbon dioxide, or the presence of uncoated BaSO_4 will diminish the difference in refractive index between the particle and the medium. A decrease in light scattering per unit surface would thus be produced, giving the material a lower obscuring power.

Since the obscuring powers were measured in water, any zinc sulfate present would be dissolved out, leaving the zinc oxide or carbonate to produce the effect. Actually lithopone *B*, having the abnormally high obscuring power, contains less than half as much zinc oxide as the others.

While too much confidence should not be placed in the linear relation of mass mean radius to obscuring power, until the effect of surface oxidation can be separated from the effect due to particle size, it serves as a good approximation. The line, if produced to the x — axis (no obscuring power), intersects it at about $550\text{ }\mu$, or $1100\text{ }\mu$ for particle diameter. This would indicate that particles of larger diameter than twice the average wave length of light have slight obscuring power, mostly specular reflection. A further study is being made of the effect of oxidation on obscuring power and also of the relation of the distribution curves to the production of minimum voids in the paint film.

Summary

1. The theory of the centrifugal method for the determination of distribution of size of particle has been discussed.
2. Results of measurements of the distribution of size of particle for six different representative lithopones have been given. The respective obscuring powers are also given.
3. An approximate relation between mass mean radius, that is, that radius that divides the mass distribution curve into equal weights of material, and obscuring power has been obtained. This relation is evidently dependent on the amount of surface oxidation as well as size of particle.

ELASTICITY AND SOME STRUCTURAL FEATURES OF SOAP SOLUTIONS

BY WILLIAM SEIFRIZ *

Introduction

Pure liquids and true solutions possess an internal friction which we term "viscosity." Such liquids, even when of high consistency, do not return to an original shape when deformed, that is, they lack rigidity and elasticity. There are, however, liquids which exhibit a pronounced tendency to recover from distortion. Solutions of certain of the organic colloids are such liquids. Gelatine, soap, and albumin are highly elastic, and this is true of the dilute liquid state as well as of the solid.

The lyophilic colloids, for the most part, possess both a high degree of viscosity¹ and of elasticity. It is the task of the colloid physicist to distinguish between these two properties, and especially to know which he is dealing with when making determinations of their values.

The time honored method of measuring viscosity values of liquids is to ascertain the time of flow of a given volume through a capillary, as compared with a standard solution. The best known apparatus for this has been the Ostwald viscometer. The method is reasonably precise for true solutions but yields irregular results when used for solutions of lyophilic colloids.

A number of investigators, notably Garrett² and Hatschek,³ have pointed out that while pure liquids and true solutions give readily duplicable results in viscosity values when these are determined with a capillary viscometer, many colloidal solutions give results which vary with the pressure at which they are drawn through the capillary. Further, the values obtained by one method (the capillary viscometer) differ from those obtained by another method (the damping of a swinging disk, or the torsion method of Couette⁴). These discrepancies in apparent viscosity values of colloidal solutions are the outcome of the elasticity or rigidity of the solution, which is, in turn, dependent upon colloidal structure.

* National Research Fellow in the Biological Sciences.

¹ Some would prefer using "plasticity" in place of "viscosity" for solutions of the (elastic) lyophiles which do not follow Poiseuille's law.

² Garrett, H., "Über die Viskosität und den Zusammenhang einiger Kolloidlösungen," Diss., Heidelberg, 1908.

³ Hatschek, E., *Koll.-Zeitschr.*, 13, 88 (1913).

⁴ Couette, *Ann. de Chim. et de Phys.* (5), 21, 433 (1890); (see also Freundlich, H., "Kapillarchemie," Leipzig, 1922, p. 744).

The influence of collapse of structure on experimentally determined viscosity values is nicely illustrated in successive viscosity readings made of a soap solution with a capillary viscometer. A liquid soap (Na-stearate) solution about four times as viscous as water, gave the following readings in seconds for the time required to fall through a one millimeter capillary — 44.2, 41.8, 39.4, 38.8, and 38.3.

Freundlich and Seifriz⁵ found that the time of fall of a shot through 30 cm. of a 0.85% concentration of commercial gelatine, which at this concentration yields a liquid gel, *i.e.*, a lumpy but readily flowable liquid, gradually decreases with each successive reading owing to a path established through the structure of the soft gel by the falling shots. The times in seconds were, 22, 14, 12, 10.5, 9.4, and 8.6.

One would be inclined to assume that the first value obtained with a capillary viscometer would be the most precise. This would be true if it were only collapse of structure with which we had to deal. But there is the other related factor, elasticity, which influences the determination. The problem of experimenters is to ascertain the extent of the influence of elasticity on apparent viscosity, and to develop a method for measuring true viscosity values. The present work owes its origin to a study of this problem by Freundlich and Schalek.⁶

Experimental

The Experiments of Freundlich and Schalek. In the determination of viscosity values of organic solutions, Freundlich and Schalek employed two types of apparatus, the Hess⁷ and the Couette⁴ viscometers. The former consists of two horizontal and parallel capillaries, through which are drawn, under suction, the standard (water or glycerine) and the unknown solutions. The Couette viscometer consists of a drum suspended by a torsion thread in the solution which is kept in motion by a revolving cylindrical receptacle. The task of Freundlich and Schalek was to determine the effect of different rates of shear (pressure in the capillary and speed of rotation in the Couette apparatus) on the viscosity values obtained, and thus learn if Poiseuille's law⁸ holds in the case of the various colloidal solutions studied, that is, if one has to do with elasticity in the solutions or not.

⁵ Freundlich, H., and Seifriz, W., Über die Elastizität von Solen und Gelen, *Zeitschr. f. physik. Chemie*, 104, 233-261 (1928).

⁶ Freundlich, H., and Schalek, E., Über die Zähigkeit und Elastizität kolloider Lösungen, *Zeitschr. f. physik. Chemie*, 108, 153-174 (1928).

⁷ Hess, W. R., *Pflüg. Arch.*, 162, 187 (1915). (See also Freundlich and Schalek, *loc. cit.*)

⁴ *Loc. cit.*

⁸ It will be recalled that Poiseuille's empirical formula expresses the relation between the viscosity value and the experimental factors involved in making measurements with a capillary viscometer.

$\eta = \frac{\pi r^4 \rho t}{8lv}$, where ρ is the pressure at which the solution is drawn through the capillary, r the radius and l the length of the capillary, t the time of fall, and v the volume of solution.

Measuring a variety of solutions Freundlich and Schalek found that "sols" of vanadium pentoxide, gelatine, and sodium stearate (Kahlbaum) deviate from Poiseuille's law, and, therefore, must be, as they are, elastic. A number of lyophobic sols, such as As_2S_3 and LaO_3 , follow Poiseuille's law, *i.e.*, they show no change in viscosity value with change in pressure. It is assumed that these lyophobic sols are inelastic.

The soap Na-stearate (Kahlbaum) exhibited that variation in experimentally determined viscosity values which was expected of an elastic sol, but the soap Na-oleate showed no such variation, *i.e.*, it followed Poiseuille's law no matter at what rate of shear its viscosity values were determined. These results of Freundlich and Schalek are in complete agreement with previously determined data of Freundlich and Seifriz⁵ who found that the Na-stearate (Kahlbaum) soap possesses a high stretching capacity in so dilute a concentration as 0.1%, with a consistency barely twice that of water, while the Na-oleate soap in a concentration as great as 40 grams in 100 cc. of water, is wholly inelastic, although the consistency is 400 times as great as that of water.

Extraordinary, however, was the difference in behavior of two Na-stearate soaps obtained from two manufacturers. In the experiments of Freundlich and Schalek a Na-stearate soap from Kahlbaum showed the expected deviations from Poiseuille's law, but a Na-stearate from Merck did not show this deviation. It was this information which was communicated to the writer. The natural assumption was that the Na-stearate from Kahlbaum is elastic (as previous work had shown to be true), and that the Na-stearate from Merck is not elastic. The writer set out to determine the truth of this assumption. The experiments not only accomplished this but brought to light some fundamental differences in physical properties of the two soaps.

Method. The object of the present investigation was to test, by means of a technique developed by Freundlich and Seifriz,⁶ the elastic properties of the two soaps. This technique was devised to make possible the determinations of elastic values of very dilute solutions. A minute magnetic particle is suspended in the colloidal solution and subjected to the influence of a magnetic field. After the particle has been attracted by the electro-magnet, the current is released and the particle returns to its original position, provided, first, that the solution is elastic, and, second, that the stretching limit has not been exceeded.

An important feature of the technique is the use of a micromanipulator by means of which it is possible to handle exceedingly minute particles. The instrument consists essentially of two manipulators each holding a glass needle which is drawn to a very fine but rigid point. The needles can be accurately controlled under the highest powers of

⁵ *Loc. cit.*

the microscope. Several makes of such instruments now exist. The one used by the writer was the Péterfi⁹ model. By means of mechanically controlled microneedles, particles as small as 5 μ can be handled. The smaller the particles, naturally, the thinner the solution can be, since the particle must remain in suspension.

The magnetic metal used is preferably nickel. The size of the particle employed in the following experiments was, unless otherwise stated, 15 μ . Particles of this size can be obtained by passing a nickel powder (from Kahlbaum) through a copper screen with 10,000 meshes per sq. cm., made of 0.035 mm. wire with a mesh width of 0.064 mm. Among the screenings are to be found 15 μ and smaller particles. The nickel particle is picked up on the tip of a gelatine or vaseline coated needle, then brought into the colloidal solution which rests in a small glass receptacle under the microscope objective, and dislodged by the second needle. Thus is the particle left suspended in the solution.

The particle must rest at least a millimeter below the surface of the solution since organic colloids have a tendency to form surface membranes of considerable resistance whose elastic values are greatly in excess of that of the interior of the solution.

With the 15 μ particle suspended in the solution, the end of a specially constructed electro-magnet is brought to within a millimeter of the particle. The current is then applied and the distance the particle is attracted, provided it returns to its original position, is measured by means of an ocular micrometer. The particle is brought, in successive steps, nearer to the magnet pole until the maximum stretching value of the solution is obtained. When the elastic limit is surpassed the particle tears through the solution and then, on release of the current, fails to return the full distance which it has traversed.

The two Na-stearate soaps used by Freundlich and Schalek were presumably of high quality, one coming from Kahlbaum and the other from Merck. For convenience they will be referred to as Na-stearate-K and Na-stearate-M.

It should be mentioned that the marked difference in behavior of the two samples of Na-stearate soaps indicates clearly that they differ in chemical constitution. There is little likelihood of either being pure, neutral, anhydrous sodium stearate.

The soap solutions were prepared by dissolving a weighed amount of soap in a definite amount of water, kept in a water bath at 70° C. for one hour, and then allowed to stand over night. The elasticity determinations were then made.

Experimental Data. A 0.2% concentration of Na-stearate-K is sufficiently rigid, though but three times as viscous as water, to support

⁹ Péterfi, T., "Das Mikrurgische Verfahren, Handbuch der mikrobiotechnik," Berlin, (1928).

a 15 μ particle. It gives a maximum stretching value of 83 μ . The same concentration of the Na-stearate-M is too thin a solution to support a 15 μ metal particle. A 0.5% concentration of N-stearate-K yields a soft, thickly flowing gel. Na-stearate-M of this concentration is still too dilute to support a 15 μ particle. A 1.0% concentration of Na-stearate-K forms a rigid gel. A 1.0% Na-stearate-M solution has a viscosity value thirty-one times that of water, which is sufficient to support a minute particle, and show a very slight stretching value (max. 8 μ).

The data are sufficient to fully substantiate the experiments of Freundlich and Schalek. At one concentration the Na-stearate-K soap forms a highly elastic gel while the Na-stearate-M forms a very viscous liquid with a barely measurable elastic value. It is due to this marked difference in elastic properties of the two soaps that Freundlich and Schalek found that the one soap from Kahlbaum showed the expected deviation from Poiseuille's law, while the other from Merck exhibited no such deviation. The following further experiments bring this fact out still more strikingly.

Relative Solubility. When the two soap powders are put into their respective flasks of water, Na-stearate-M immediately forms a fine stable colloidal suspension with the opacity of thin milk, while Na-stearate-K goes into dispersion very poorly. On the other hand, at a temperature of 60° C. the Merck soap dispersion is still chalky in appearance, while the Kahlbaum soap is a quite clear solution. No difference in degree of transparency between the hot Na-stearate-K solution and pure water could be noticed. Apparently we are dealing here with a molecular dispersion of the Kahlbaum soap. Undoubtedly the ability of the Kahlbaum soap to form a true solution at 60° C. is intimately associated with its elastic properties when cold, as compared with the absence of both these characteristics in the Merck soap. On cooling, the Na-stearate-K becomes as opaque as Na-stearate-M—colloidal structure has arisen out of the molecular dispersion.

In still another respect the two soaps are strikingly different. When cold and dilute the Kahlbaum soap shows a fibrous appearance which can best be described by noting its resemblance to spun glass or asbestos wool. This appearance is not characteristic of the Merck soap. The marked spun glass or asbestos wool appearance of the Kahlbaum soap points very decidedly to a fibrous structure, with a corresponding absence of this structure in the Merck soap. Microscopic examination substantiates this.

Microscopic Structure. Both soaps were subjected to microscopic examinations, the results of which are best given by reference to photographs.

Photographs Nos. 1-6 represent a magnification of 75 diameters, No. 7 of 100, and No. 8 of 500.

Photograph 1 (Plate 1) is of Na-stearate-K, depicting the microscopic structure of a solution of low concentration (0.2%) which flows freely and smoothly, is quite elastic, and of low consistency. The picture shows something of the denser streaks which give to the solution its spun-glass or asbestos-wool-like appearance. Especially evident is the fibrous nature of the particles which make up the system. The average length of the readily visible rod-shaped particles is about 0.04 mm. Greater lengths, with a maximum of 0.08 mm., are not infrequent. The finest of the long, tenuous fibers have an estimated thickness of $0.5\ \mu$ and are visible only with high powers of the microscope.

In contrast to the Na-stearate-K solution is that of Na-stearate-M shown in photograph 2. There is here no indication of a fibrous structure. The picture presents the usual typical chalky appearance of this inelastic soap.

Photograph 3 illustrates the early stages of curd formation in Na-stearate-K. In the photograph are some seven curd nuclei. The largest is at the left edge of the photograph and the smallest immediately below (to the left). The curd nuclei are built up of fibers much finer than the free floating ones. They commence growth with a coarse particle of soap as a center. About this center, or "nucleolus," is the curd nucleus formed. A nucleolus, or center of curd formation, is to be seen in the young curd growth at the extreme left, and again in two other nuclei in the lower left center of photograph 3.

In photograph 4 is shown part of a larger curd mass with its crystalline, fibrous structure. This nucleus measured in total length 1.4 mm. The solution is a Na-stearate-K soap of 0.45% concentration which forms a soft, thickly-flowing gel.

The delicate nature of these curd nuclei is best illustrated in photograph 6. (Plate 2.) A minute nucleus is at the upper edge. Its delicate, faint fibers can be seen radiating from both ends of the nucleolus. The center of the photograph is occupied by a large curd mass.

It will be noticed that the growth of the curd nucleus is greater in one direction than in the other so that the crystalline mass soon assumes a linear configuration. This is clearly shown in the curd nucleus protruding above the lower edge of the photograph. The linear axis of this nucleus lies parallel to the lower edge of the page.

The marked polarity of the curd nuclei is well illustrated in the young, small nucleus at the top of photograph 6. It would seem that polarity is already present in the nucleolus before growth starts, since the few fibers so far formed are attached to opposite ends of the nucleolus with as yet no fibrous outgrowths at the sides. Polarity, then,

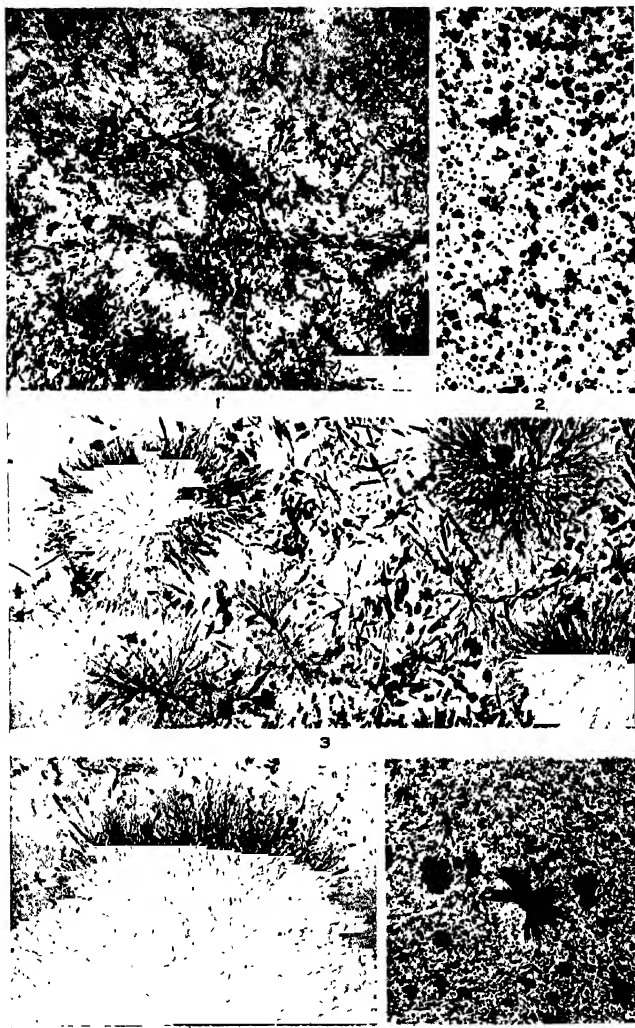


PLATE 1.

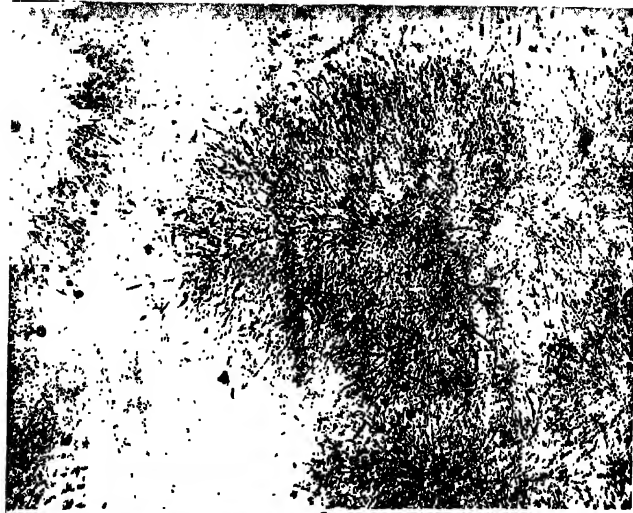


PLATE 2.

which manifests itself in an axis of greater growth, is established when the first fibers of the crystalline soap curd are laid down.

The fibers which build up the curd are seen to be much longer and infinitely more delicate than are the readily visible, coarse, free-floating rods. This is best illustrated in the radiating fibers of the young gel nucleus lying at the upper edge of photograph 6. The fibrous strands of this small nucleus measure about 0.1 mm. in length. The free-floating, rod-shaped particles, which appear in photograph 1, play no part in the building up of the ultimate curd structure of the soap. The structural units from which the curd mass is built are submicroscopic in size, *i.e.*, colloidal or molecular. From these are formed the microscopically visible, tenuous, crystalline fibers of the curd.

Photograph 5 illustrates the maximum degree of fiber and curd formation in a Na-stearate-M soap. One single small cluster of fibers and a few scattered fibrous particles are all that are visible, and is as far as the Na-stearate-M soap ever gets toward curd formation.

The visible curd masses of the Na-stearate-K soap possibly do not represent the actual colloidal structure of the soap. They have rather crystallized out of the colloidal solution. But they do suggest a fibrous nature of the elastic gels, since they appear only in elastic soaps and not in inelastic ones.

Sodium oleate is an extraordinary intermediate case. This soap in these experiments never exhibited any marked elasticity, yet the solution possesses numerous, free floating fibers suspended in the otherwise granular matrix (Photo 7). These crystalline fibers are often of great length, the maximum being 0.166 mm.

The Possible Influence of H-ion Concentration. From the extensive work that has been done on the effect of acidity on the viscosity of gelatine solutions, it is natural to suspect that pH may be a, if not the, determining factor in the behavior of the Kahlbaum and Merck soaps.

The pH of the Kahlbaum soap is somewhat higher than that of the Merck soap. An eighteen hour old solution of Na-stearate-K has a pH of 9.55, while a Na-stearate-M has a pH of 9.1. Furthermore, with time, due to adsorption of CO₂, there is a drop in pH (to 8.6) and a corresponding lowering of stability of the soaps. Ultimately, both soaps are precipitated out. But the Kahlbaum soap may fall to the pH of the Merck soap and still show gel characteristics. H-ion concentration determines stability but it does not appear to be a deciding factor in the formation of gel structure and consequent elasticity. One cannot have low pH and high stability, but one can have stability, due to high pH, without rigidity and elasticity.

Further evidence against acidity as a factor determining elastic properties of a soap solution, is to be had from samples of inelastic Na-oleate solutions to which gradually increasing amounts of NaOH are

added. The increased alkalinity greatly raises the consistency of the soap solution but adds nothing to its elasticity.

Addenda

The above account closes the experimental work on the two sodium stearate soaps originally obtained by Freundlich and Schalek from Kahlbaum and Merck. No more of these original samples are available. Other samples of Na-stearate purchased from Merck, both before and after that obtained by Freundlich and Schalek are elastic. The microscopic structure, however, is strikingly different from that of the elastic Na-stearate-K soap. No curd masses of typical crystalline nature are to be observed. Instead, the soft elastic soap is, in its microscopically visible structure, a mass of entangled fibers (Photo 8) some of which are of exceedingly great length (maximum actually measured was 0.55 mm.).

These marked differences in structural features are undoubtedly due to impurities in the soap. Yet, even realizing this, it is extraordinary that such variety in structure is obtainable in soap solutions whose chemical constitutions are predominantly the same.

Ammonium Oleate. After the completion of the foregoing work, the writer's attention was called by Mr. Hatschek to the elastic property of ammonium oleate, together with an ingenious method of demonstrating it. With Mr. Hatschek's kind permission the following statement, from as yet unpublished data, is made.

Very dilute solutions of ammonium oleate exhibit pronounced elasticity which can be demonstrated in a very simple manner.

Five cc. of strong ammonia water (28% concentration, sp. gr. 0.9) is diluted to 100 cc. and to this is added, one drop at a time with 4-5 minute intervals, some 5 drops of oleic acid. After each drop the solution is thoroughly mixed by rapidly rotating it in a flask. Usually, after the second or third drop the elasticity of the solution is clearly evident from the fact that when movement in the one direction, due to giving the flask a rapid rotating motion, stops, instead of the liquid remaining still there is a pronounced return movement which may be as great as 180° . The phenomenon is prettily demonstrated by draining off the perfectly clear thin solution, free from undissolved soap, from a burette. The few air bubbles formed by the rotation suffice to serve as indicators of the return movement.

The writer wishes to add the following observations to those of Mr. Hatschek.

Heating to 60° C. destroys all evidence of elasticity in a dilute NH_4 -oleate solution, both while hot and after cooling again. This may be due to driving off of the NH_4 from the $\text{NH}_4\text{C}_{18}\text{H}_{35}\text{O}_2$ molecule—

though one would hardly expect this to be complete at 60° C.—or to some physical change as a result of heating.

If several cc. of oleic acid are added to 100 cc. of 5% (5 cc. of 28%) ammonia water, rotating in a flask will yield an exceedingly elastic and viscous gel. Magnetically, an elastic limit of 450 μ is obtained—the highest yet observed in any elastic organic substance. Coarse macroscopic strands of this soap may be stretched several inches.

The highly elastic state of the NH_4 -oleate gel just described is typical immediately after vigorous rotating. In a few minutes' time this elasticity may be completely lost, and the viscosity become so low that it is barely sufficient to support a 10 μ nickel particle. If such a particle, while suspended in a concentrated NH_4 -oleate solution which has stood a few minutes, is magnetically attracted it shows little or no return movement. The stretching capacity is zero.

If, now, this wholly inelastic solution of NH_4 -oleate is again given one or two vigorous rotational swings in a flask, it quickly assumes the very high elasticity it formerly possessed. Mere pouring of the viscous gel from the flask clearly indicates its extreme elastic state. After the solution has stood a short time, it is again seen to be quite inelastic when slowly poured a drop at a time. The process may be repeated again and again even after several days.

No attempt will be made here to interpret this unusual phenomenon except to point out, what is quite evident, that a colloidal structure is built up by agitation of the solution and then collapses on standing. An interesting relation exists between this phenomenon and two similar ones described by Schalek and Szegvary¹⁰ and Svedberg.¹¹ In these latter two cases the colloidal structure is built up on standing of the solution and broken down by mechanical agitation.

Schalek and Szegvary's experiment consisted in adding a small amount of electrolyte to a 6-10% iron oxide solution. Gelation follows in time, a soft, poorly elastic, transparent jelly resulting. Mere shaking of the vessel will cause the gel to liquefy and the thin sol gels again on standing. The process may be repeated many times.

Svedberg's experiment was similar. A metallic cadmium solution is prepared in alcohol by electric pulverization. In time the solution sets into a very firm jelly. Stirring with a rod causes complete collapse, and the newly formed solution is no more viscous than was the original alcoholic sol.

In the inorganic solutions of Schalek and Szegvary, and of Svedberg a building up of colloidal structure results from standing, while in the organic NH_4 -oleate soap above described, gel formation results from

¹⁰ Schalek, E., and Szegvary, A., *Ueber Eisenoxydgallerten*, *Koll.-Zeitschr.*, **32**, 318-19 (1920).

¹¹ Svedberg, T., Discussion on the physical properties of elastic gels, *Rep. Faraday Soc. and Phys. Soc. of London on Phys. and Chem. of Colloids*, 1-13 (1921).

mechanical agitation. No microscopically visible structure exists in the elastic gel of ammonium oleate. Lines of stress in the gel are the only indication of a continuous structure, nor is there any curd formation.

Ultramicroscopically, the particles of the NH_4 -oleate soap, when in the inelastic state (the collapsed condition after standing) are spherical. The minute ultramicroscopic spherites are in active Brownian movement. No ultramicroscopic picture of the elastic gel of this soap could be obtained.

The ultramicroscopic particles of the elastic Na-stearate soap in solution are short, fat rods. The ultramicroscopic particles of the inelastic Na-oleate soap in solution are spherites. McBain¹² and his co-workers have described similar ultramicroscopic fibrous structures of soaps.

It is difficult to visualize the structure of an elastic substance whose structural units are spherical, if such exists. The more minute colloidal particles are, the less easy is it to determine their shape ultramicroscopically, and the colloidal particles of the collapsed inelastic NH_4 -oleate solution are very small.

The NH_4 -oleate solution, with spherical colloidal particles (when in the collapsed state), stands as an exception to the rule that elastic solutions have rod-shaped colloidal particles. Freundlich and Seifriz⁵ found Na-stearate, vanadium pentoxide, and benzopurpurin to be elastic. All three have rod-shaped colloidal particles (and are doubly refractive). They further found that Fe_2O_3 and Na-oleate are inelastic (magnetically determined). These two substances do not have fibrous colloidal particles (the Fe_2O_3 solution is also not doubly refractive).

Experimental data tend more and more to support the conception of a fibrous structure for elastic jellies, whether the fibers are colloidal, or even at times perhaps microscopic, or whether merely molecular chains as is believed by some to be true of gelatine.

Summary

1. Of two samples of Na-stearate from different manufacturers, Freundlich and Schalek found that one of them deviated from Poiseuille's law while the other followed the law. The author of the present paper has shown the former Na-stearate soap to be elastic, both as "sol" and as gel, while the latter is inelastic.

2. The elastic Na-stearate solution has rod-shaped particles (microscopic and colloidal). From the elastic solution, curd masses crystallize out. The inelastic Na-stearate solution possesses spherical particles. No curd crystallizes out of this soap.

¹² Darke, W. F., McBain, J. W., and Salmon, C. S., *The Ultramicroscopic Structure of Soaps*, *Proc. Roy. Soc., A*, **98**, 395-409 (1921).

⁵ *Loc. cit.*

3. Na-oleate is also inelastic. The ultramicroscopic particles of Na-oleate are spherites, and the greater number of the microscopically visible particles are also spherical, but numerous long crystalline rods are present. These last may become 0.16 mm. in length. This soap does not gelatinize in any concentration at room temperature.

4. Ammonium oleate, prepared after the manner of Hatschek, forms a highly elastic dilute solution. A concentrated NH_4 -oleate solution may be formed which is inelastic when in the sol state, and highly elastic as a gel. The elastic gel is formed from the inelastic sol merely by agitation, but is very unstable, soon reverting to the inelastic sol condition.

In conclusion, the writer wishes to express his indebtedness to Professor Rodney True of the Department of Botany of the University of Pennsylvania for the purchase of apparatus and for placing the facilities of his department at the writer's disposal.

University of Pennsylvania,
Philadelphia, Pa.

A SIMPLIFIED SLIT ULTRAMICROSCOPE

BY L. V. FOSTER

The need for a simple portable slit ultramicroscope among the colloid chemists was first brought to our attention by Dr. Jerome Alexander.

The slit ultramicroscope is a rather recent development having been invented by Dr. Seidentopf and Dr. Zsigmondy in 1900. The general form of the instrument which they devised has been little changed since that time. It consisted of a rather complicated arrangement of illumination system, observing microscope and object holder. The instrument was extremely large and occupied considerable space in a laboratory. To put the apparatus in adjustment required several hours of careful work by an experienced microscopist. This paper deals with a slit ultramicroscope, designed by the author, which is portable, self-contained and requires little effort to adjust for use.

Before describing this simplified slit ultramicroscope I will point out briefly the capabilities and limitations of the ordinary compound microscope. Of course, the reason for applying the microscope to the study of any object is because the naked eye cannot see all the details existing within the object. Everybody knows that a magnifier is a great help to the eye in observing fine structure such as the grain in wood, the fibers in paper, and cloth, or finely divided scales. When one looks through the magnifier at such objects, the detail, which the eye cannot see, is enlarged and separated and becomes visible. Figure 1 shows a picture of cloth as the eye would see it without a magnifier. Figure 2 shows the same cloth under a magnification of 5X.

But there is detail which one cannot see with the magnifier. More magnification and more resolution is required. The microscope has been developed for such purposes, but it also has a limit, beyond which structure cannot be separated. Resolution with the microscope objective depends upon numerical aperture. The greater the numerical aperture, the greater the resolution. Figure 3 shows the meaning of numerical aperture which is expressed by the product $n \sin \alpha$ where n is the index of refraction of the least refracting medium between the object and the front lens of the objective, and α is one half the total angle taken in by the front lens of the objective. It can easily be seen that in order to obtain maximum resolution, both $\sin \alpha$ and n must be a maximum. The limit for $\sin \alpha$ is 1.0 when α is 90° and the maximum for n is about 1.7 in the visual spectrum. Microscope objectives have been made for visual work with numerical aperture as great as 1.60, none have been

of greater numerical aperture. The limit of resolution for an objective having a numerical aperture of 1.60 in the visual spectrum is equal to about 0.13 microns.

Further explanation is necessary to show what bearing numerical aperture has upon resolution. This relation was discovered by Pro-

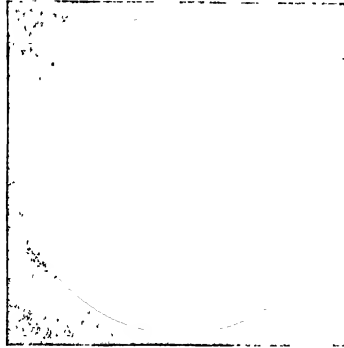


FIG. 1.—Cloth. Natural size.

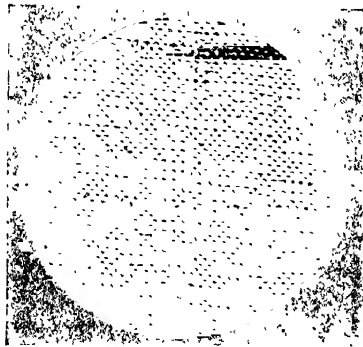


FIG. 2.—Cloth. Magnified 5 \times .

fessor Abbe, and is clearly set forth in the Proceedings of the Bristol Naturalist's Society New Series Vol. I, Part II (1874-5) where a translation by H. E. Fripp of Professor Abbe's "Contribution to the Theory of the Microscope, and the Nature of Microscopic Vision" appeared. For the sake of those to whom this article is not available, I will state the conclusions drawn by Professor Abbe. Light always suffers dif-

fraction at an opaque edge. A series of opaque lines, such as the markings on diatoms, give rise to a series of diffraction spectra and the separation of these spectra depends upon the separation of the markings. Figure 4 is a schematic diagram of a grating and a microscope objective. The grating is illuminated by a narrow cone of light which is shown in full lines. At the grating diffraction takes place and the first dif-

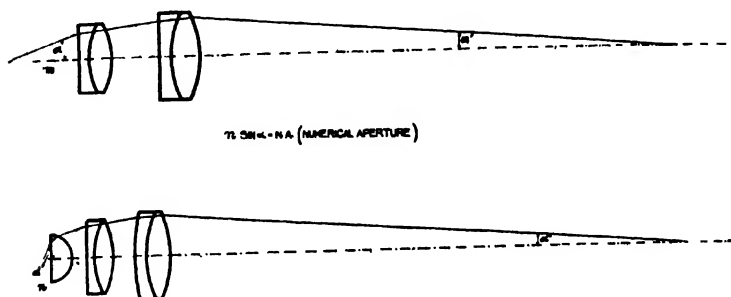


FIG. 3.

fraction spectrum coming from the object is shown in dotted lines. These two cones of light pass through the objective and are brought to focus by it in the upper focal plane. In order to be able to discern the structure of the object which gives this diffraction image, the numerical aperture of the objective must be large enough to pass both of these pencils of light which unite at the upper focal plane of the objective to form the image. Therefore, for the resolution of structure, the

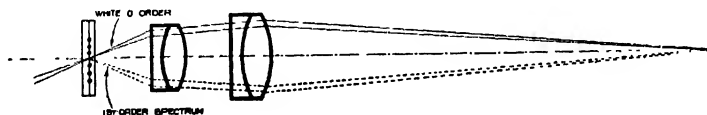


FIG. 4.—Grating and microscope objective.

microscope objective must receive central illumination through the object and at least the first diffraction pattern which arises from this object.

The slit ultramicroscope goes one step further than the ordinary microscope of highest resolving power. In ordinary transparent microscopy, particles separated by less than 0.13 microns cannot be seen as separate individual particles. This, of course, can be explained by the fact that the distance between the particles is of such a size that the diffraction pattern which arises makes an angle with the axis of the cone of light illuminating the particles greater than the full angle which

can be received by the objective. Thus, the particles are not seen as separate but may be seen as one provided that two or more taken together are of a size greater than 0.13 microns. Such particles if illuminated with strong light and observed against a black background will diffract light in all directions and will be seen as self-luminous objects. It is not possible to get this effect in ordinary transparent illumination. Dr. Siedentopf working with Dr. Zsigmondy, in 1900, found that by illuminating such colloidal solutions as soap, gold, silver, and rosin, with extremely strong light, and observing them through a microscope

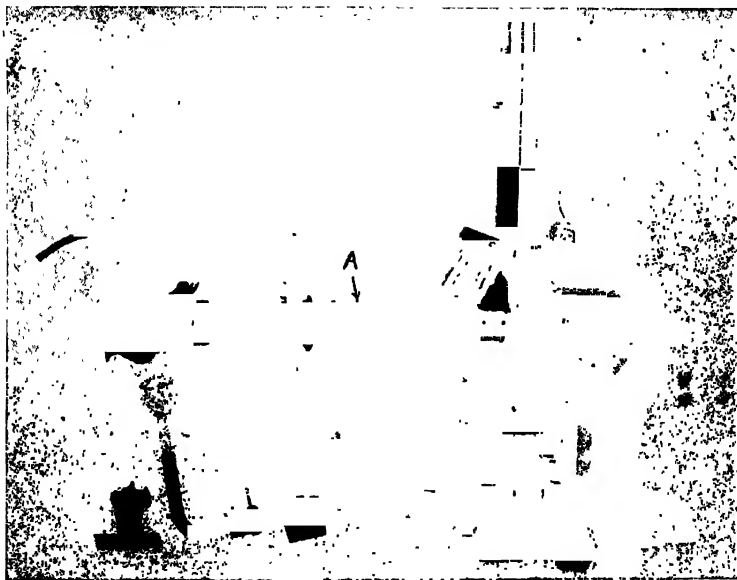


FIG. 5.—Slit ultramicroscope for the examination of gases.

located with its axis 90° to the axis of the illuminating system, was able to see numerous points of light which he called "ultramicroscopic particles" made self-luminous against a black background by this extremely strong illumination.

The writer has designed a slit ultramicroscope which is simple to operate and can be used for observing colloids in liquids, gases and solids. Figure 5 is an illustration of the microscope arranged for the observation of colloids in gases. Beneath the microscope in the same illustration is an adjustable table for holding solids for examination. This table replaces the gas chamber shown on the stage of the micro-

scope. The liquid chamber is shown in the same illustration and consists of four separate parts, which fit together with ground tapered tubes. The description of this is given in the paragraph dealing with the examination of liquids. The slit, illumination system, and objective, for imaging the slit are all mounted in one tube which is fastened to a mechanical stage having transverse and longitudinal movements. When the light, a mazda lamp, is centered and focused in the slit, the complete unit can be racked to a position such that the axis of the illumination

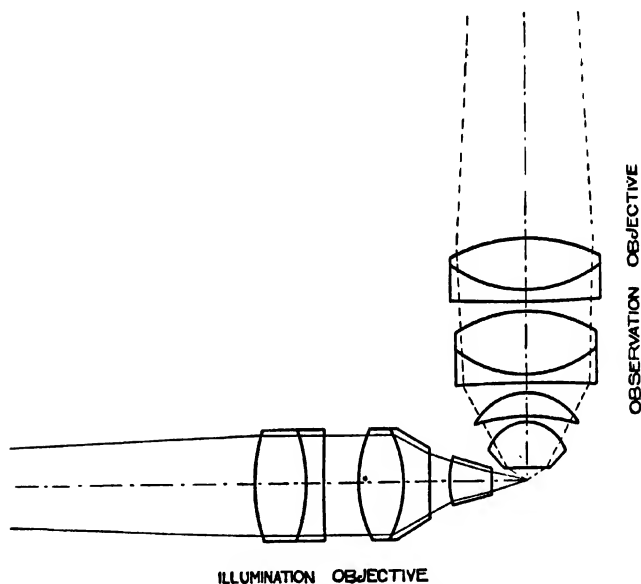


FIG. 6.—Position of the illumination and observation objectives when adjusted for ultramicroscopic examination of liquids.

pierces the axis of the observing microscope. The slit ultramicroscope is then ready for use in the examination of either solids, gases, or liquids. The observing microscope, in the case of solids, and gases, is an ordinary compound microscope consisting of a 16 mm. objective and any eyepiece desired. The illumination system, located 90° to the observing microscope, consists of a light source imaged by a condensing system in a very narrow slit which is placed in a horizontal position in the tube *A*. The slit is imaged by another 16 mm. objective in the object plane of the observing microscope.

The microscope becomes somewhat more complicated when it is to be used for the examination of colloidal particles in liquids. In order

to make use of a mazda lamp for observing colloidal particles in liquids, it is necessary to utilize as much illumination from the source as possible. A water immersion objective of N. A. 0.50 was designed especially for the purpose of condenser to form a very intense image of the illuminated slit in the liquid. The observing objective was also made with very high numerical aperture in order to gather as much diffracted light from the colloidal particles as possible. The numerical aperture of this objective is N.A. 1.0. To get these high numerical apertures and be able to get a condensing objective and an observing objective close

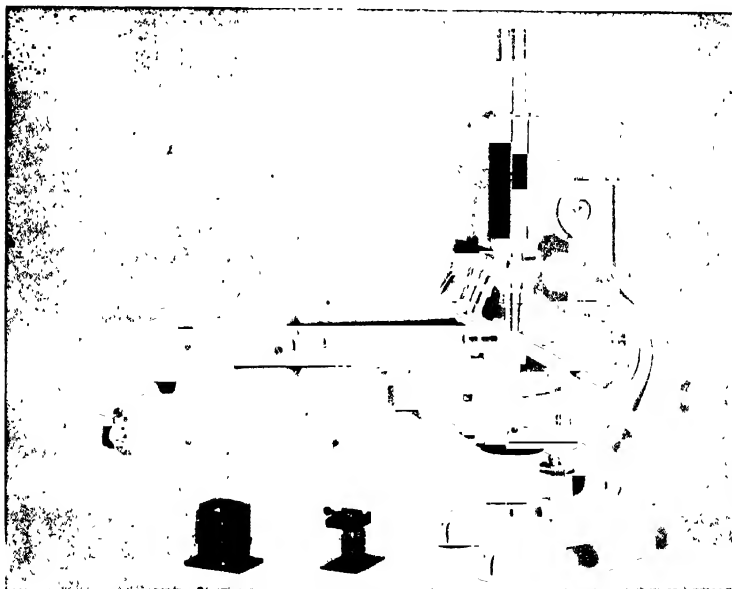


FIG. 7.—Slit ultramicroscope for the examination of liquids.

enough so that their object points coincide required constructing them with very long working distances. The condensing objective was made 7.0 mm. focal length with 2.0 mm. working distance and the observing objective was made 4.0 mm. focal length with 0.6 mm. working distance. Figure 6 shows the manner in which these objectives have been made so that their object points coincide. After getting a condenser and objective having the above described properties, a liquid cell had to be designed which would contain the front lenses of the condenser and objective and permit focusing and still be water-tight. The liquid cell was constructed of hard rubber and has a section of vulcanized rubber

attached to it provided with special openings to admit the condensing and observing objectives. The liquid cell was fastened to the condensing objective by means of a clamp so that this objective automatically centers itself in the chamber. The observing objective can then be focused into the opening in the top of this chamber until the colloidal particles illuminated by means of the illuminating system come into focus. A thistle tube and connecting tube are attached to the end of the chamber containing the stop cock allowing one to retain a reservoir of liquid and observe different portions of it at will. The thistle and connecting tubes must be attached to the end containing the stop cock in order to hold back the column of liquid so that its weight will not force the liquid out through the connection between the chamber and objectives. Figure 7 shows the apparatus set up for liquids.

When the microscope is in adjustment for either solids, gases or liquids the slit in the illumination system may be rotated to a vertical position and the length of it shortened to 2 mm. when it will be possible to observe the thickness of the layer under examination. This thickness may be increased or decreased by means of the knurled head screw which opens and closes the slit.

This instrument has been designed with the aim that it be made a simple but efficient instrument for ultramicroscopic observations. The illumination system has been mounted in a tube fitted so that it can be attached to any mechanical stage. The mechanical stage can be removed from the microscope and placed at the front of the stand so that the illumination tube overhangs the front of the microscope, giving it great stability. The axis of the illumination system can be adjusted easily for a central position by means of the transverse movement on the mechanical stage and can be brought into focus in coincidence with the axis of the observing microscope by means of the longitudinal movement on the mechanical stage.

Scientific Bureau of Bausch & Lomb Optical Co.,
Rochester, N. Y.

THE PLASTICITY OF RUBBER AND ITS SOLS. I

By W. J. KELLY

During the past several years considerable work has been done on the "viscosity" of rubber sols, principally in benzol, for the purpose of discovering a possible relation between this and the other physical properties of the rubber after vulcanization. Originally it was thought that the viscosity would be a measure of the degree of polymerization of the rubber and as such would indicate the tensile strength of the vulcanized sample. However, it was found by de Vries¹ and his collaborators that the effect of the non-rubber substances such as resins, sugars, etc., had a very great effect on the measurements of viscosity as did also other substances such as acetic, hydrochloric and sulfuric acids, ammonia sodium hydroxide, and many others when added in very small amounts to a benzol sol. Some of these reduce the viscosity whereas others, notably ammonia and sodium hydroxide, increase it. The effect of moisture in the sample is very great as rubber dried for long periods over sulfuric acid or quick lime showed, in some cases, nearly a threefold increase in relative viscosity, that of benzol at the same temperature being taken as unity. It is, in view of the effect of other agencies, practically impossible to find any relationship between viscosity and other physical properties. Hence, it seems highly desirable to study the effect of these agencies on the viscosity of rubber sols in the light of present ideas as to the structure of rubber.

Methods of Measurement

In practically all of the work done in the past the Ostwald or similar viscometer has been used and the viscosity relative to that of the solvent taken as an index of the viscosity of the sol. It has been stated by Herschel² that sols of unmilled rubber do not obey Poiseuille's Law and are, therefore, not viscous but plastic. Such being the case it could hardly be expected that a determination of the relative viscosity of a rubber sol at a single shearing stress would give any information as to its behavior under other conditions. Herschel also states that rubber which has been milled gives a sol which does obey Poiseuille's Law when the concentration is one or two per cent, and that as the time of milling is prolonged the viscosity decreases. This has also been

shown by other writers. Herschel, however, gives no data as to time and temperature of milling nor for the viscosity of the sols. According to de Vries two Ostwald viscometers having capillaries of different bore will not give the same relative viscosity, but that the one with the larger bore shows a lower value. This is exactly what one would expect to find in dealing with a substance which does not obey Poiseuille's Law, or in other words, which is plastic.

Therefore, in starting the work to be presented in these papers it was decided to employ an instrument which would give a series of shearing stresses so that the complete relationship between this and the rate of shear could be followed easily. Hence it was decided to use the Bingham-Murray "Combined Viscometer and Plastometer."³ Since some authorities, notably Green,⁴ have raised serious objections to the results obtained by this method, it seemed desirable to make a study of a viscous substance in tubes of different radii and to find the method of calculating the results which would give the most accurate and reliable value of the fluidity. For this purpose castor oil was chosen because its consistency is approximately that of several of the rubber sols which were to be studied.

In this paper only the results of the work on castor oil will be presented in detail and the line of attack on the rubber sols indicated. The reason for this is that although the laws governing viscous flow in capillary tubes are definitely known, those governing plastic flow are not so general and are known for only a comparatively few systems. Rubber sols do not belong to the systems of which the laws are known and although considerable progress has been made the results are not as yet sufficiently convincing to warrant publication at this time.

Effect of Surface Tension

Among the objections that have been raised against the Bingham-Murray method of measuring viscosity or plasticity is the one that the surface tension of the liquid being measured exerts an unknown and undeterminable pull on the column of liquid in the tube and hence introduces an error into the determination. There is no doubt that this is the case but the pull due to the surface tension can be approximated very closely, and when added to the pressure gives the total pressure causing flow in the tube within limits which can be determined easily.

The magnitude of this pull is given by the equation

$$hd = \frac{2S \cos a}{Rg} \quad (1)$$

where h is the height to which the liquid would rise in a tube of radius R , S the surface tension, a the contact angle, d the density of the liquid

and g the gravitational acceleration. If the liquid is flowing into a tube which is already wet with the liquid the value of a is zero and hence, $\cos a$ is unity. On the other hand, if the tube is dry it takes a certain time for the contact angle to establish itself and hence the greater the velocity of the meniscus the greater will be the contact angle and the smaller $\cos a$. This means that the pull exerted by the surface tension is a variable. It is possible to force the liquid through the tube at a velocity great enough to make the angle of contact greater than 90° , in which case the surface tension would give rise to a back pressure in the tube. In none of the experiments recorded here has any been observed where the meniscus was not concave and hence the contact angle less than 90° . Such being the case, we can put an upper limit on the correction due to surface tension. The lower limit cannot be determined easily and will vary with the velocity. If an accuracy of 1% is desired it will be necessary to work with an applied pressure at least 100 times as great as the effect of the surface tension at its maximum. For the work recorded here the surface tension correction has only been applied in cases where the applied pressure was less than 100 times the magnitude of this correction. Furthermore, at velocities permitting accurate measurement of the time the linear velocity of the meniscus must be small and hence the contact angle is probably very close to zero requiring the maximum correction to be applied.

Method of Calculating Results

Bingham and Murray calculate the volume rate of flow as the volume of the tube between two graduations divided by the time required for the meniscus to travel from the first to the second or v/t . For this method of measuring viscosity the volume rate of flow is properly expressed as $\Delta v/\Delta t$, and in this paper it is always so given except where the instantaneous rate dv/dt is used. Green⁴ objects to the use of $\Delta v/\Delta t$ as the proper value of the rate of shear, but it will be shown below that this is the correct method for viscous flow.

$$F = \frac{l}{l_2 - l_1} \int_{l_1}^{l_2} \frac{PR}{2l} dl$$

$$F = \frac{PR}{2(l_2 - l_1)} \ln \frac{l_2}{l_1} \quad (2)$$

If F is plotted against $\Delta v/\Delta t$ for either viscous liquids or plastic systems tubes of different radii will give different lines, but if F be multiplied by $\pi R^3/4$ all the lines for viscous flow will give a single straight line, passing through the origin, whose slope is the fluidity of the liquid.

For some rubber sols single curves have been obtained with different tubes but in many cases the curves do not coincide.

When F is multiplied by $\pi R^3/4$ the result is equivalent to the constant of Poiseuille's Law $\pi PR^4/8l$.

An objection to Bingham and Murray's method of calculation⁴ has been raised on the ground that whereas they were over careful in calculating F according to equation (2) they were not careful enough in calculating $\Delta v/\Delta t$. The most satisfactory way to calculate fluidities from data obtained in a Bingham-Murray tube would be to determine the instantaneous rate of flow at any given point in the tube and divide this by the value $\pi PR^4/8l$ at the same point.

To do this it is necessary to find the equation connecting v and t and this can be done as follows: For any constant volume viscometer Poiseuille's Law is

$$\frac{v}{t} = \frac{\pi PR^4}{8l} \phi \quad (3)$$

where ϕ is the fluidity of the liquid. For a given point in the Bingham-Murray instrument, this equation becomes

$$\frac{dv}{dt} = \frac{\pi PR^4}{8l} \phi \quad (4)$$

However, in order to calculate dv/dt it is necessary to transform the equation so that dv/dt can be expressed in terms of t , which can be done very easily as follows:

$$\text{Substituting in (4) } l = \frac{v}{\pi R^2}$$

$$\frac{dv}{dt} = \phi \frac{\pi^2 R^6 P}{8v}$$

or

$$2v dv = \phi \frac{\pi^2 R^6 P}{4} dt \quad (5)$$

Integrating this equation between $v_1 = 0$ and $v_2 = v$ and $t_1 = 0$ and $t_2 = t$ we get

$$v^2 = \phi \frac{\pi^2 R^6 P}{4} t$$

or

$$v = R^3 \left(\phi \frac{\pi^2 P}{4} \right)^{\frac{1}{2}} t^{\frac{1}{2}} \quad (6)$$

For any given experiment $\phi \frac{\pi^2 P}{4}$ is constant but as no capillary is absolutely uniform in bore, R^3 cannot be considered as constant. Hence we can write

$$v = KR^3 t^{\frac{1}{2}} \quad (7)$$

and

$$\frac{dv}{dt} = 1/2 KR^3 t^{-1/2} \quad (8)$$

The variation in R^3 is not very great but is still enough to cause a drift in the value of KR^3 and hence a drift in the value of the fluidity if calculated on the assumption that R^3 is constant. When plotting $\log v$ against $\log t$ the variation in R is not great enough to be noticed but as soon as the values of ϕ are calculated from the data directly the drift becomes evident. However, if the change in the value of R^3 is taken into account there is no drift in the value of ϕ as calculated by this method as can be seen from the data given below on castor oil.

There is still another factor entering into this calculation which must be taken into account. If we plot v against t , the observed time, on logarithmic paper it will be found that the line is not straight as it should be, practically, from a consideration of equation (8). Moreover the curvature is greater than would be expected from the variation in R^3 . This is due to the fact that whereas v is measured from the end of the tube t is measured from any convenient point, 5, 10 or 15 cm. from the end. If equation (8) holds then we should be able to calculate the time required for the meniscus to travel from the end of the tube to the point where the measurement of the time began. By adding this correction to the observed time and plotting this sum against v on logarithmic paper we should get a straight line except for any deviation due to the changing value of R^3 .

From Equation (8) we can write

$$v_1 = KR^3 T_1^{1/2} \quad \text{and} \quad v_2 = KR^3 T_2^{1/2}$$

where T_1 and T_2 represent the corrected times or $(t_r + t_o)$ when t_r is the observed time.

By division

$$\frac{v_2}{v_1} = \frac{R_2^3}{R_1^3} \left(\frac{T_2}{T_1} \right)^{1/2}$$

or

$$\left(\frac{T_2}{T_1} \right) = \left(\frac{v_2 R_1^3}{v_1 R_2^3} \right)^2$$

Since $T_1 = (t_{r1} + t_o)$ and $T_2 = (t_{r2} + t_o)$ we can write

$$\frac{(t_{r2} + t_o)}{(t_{r1} + t_o)} = \left(\frac{v_2 R_1^3}{v_1 R_2^3} \right)^2$$

or

$$t_o = \frac{t_{r2} - a t_{r1}}{a - 1} \quad (9)$$

where $a = \left(\frac{v_2 R_1^3}{v_1 R_2^3} \right)^2$

In Fig. 1 the lines obtained by plotting $\log v$ against $\log t_r$ and $\log v$ against $\log T$ are given. The former are decidedly curved whereas the latter are straight.

This method of calculation is rather laborious and the fact that a correction must be made to the observed time makes it less desirable

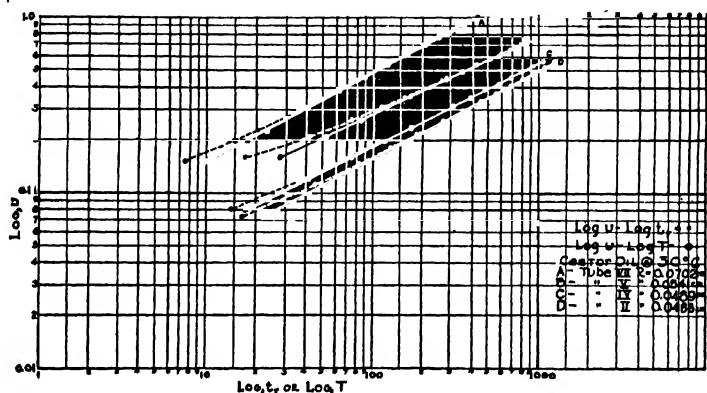


FIG. 1.

Log. v vs. Log. t_r .
Log. v vs. Log. T .

than one where no correction at all is necessary. A further consideration of Poiseuille's Law will show that it is possible to obtain an equation which is free from these objections.

If Equation (5)

$$2v dv = \phi \frac{\pi^2 R^6 P}{4} dt$$

is integrated between v_1, v_2 and t_1, t_2 we get

$$(v_2^3 - v_1^3) = \phi \frac{\pi^2 R^6 P}{4} (t_2 - t_1) \quad (10)$$

Substituting $\pi R^2 l$ for v

$$(\pi R^2)^3 (l_2^3 - l_1^3) = \phi \frac{\pi^2 R^6 P}{4} (t_2 - t_1)$$

or

$$(l_2^3 - l_1^3) = \phi \frac{PR^3}{4} (t_2 - t_1)$$

$$\frac{(l_2 - l_1)}{(t_2 - t_1)} = \phi \frac{PR^3}{4(l_2 + l_1)}$$

By substituting $\frac{v}{\pi R^2}$ for l on the left hand side of this equation

$$\frac{\frac{1}{\pi R^2}(v_2 - v_1)}{(t_2 - t_1)} = \phi \frac{PR^2}{4(l_2 + l_1)}$$

or

$$\frac{\Delta v}{\Delta t} = \phi \frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)} \quad (11)$$

Hence, the correct way to use $\Delta v / \Delta t$ is to plot it against the value of $\pi PR^4 / 8l$ calculated for a point midway between two successive graduations or to divide the former by the latter to calculate the fluidities for different intervals in the tube. The value of R must be taken as the average radius from the end of the tube to the point at which the calculation is made. If $\pi PR^4 / 8l$ is used as indicated above the correct corresponding value of the rate of flow is the slope of the tangent to the v/t curve at a point midway between the two values of v .

Equation (6) may be written

$$v^2 = (KR^3)^2 T$$

Assuming that R is constant this equation may be written

$$v^2 = K_1 T \quad (12)$$

which is the equation of a parabola. The slope of this parabola at any point v , T , is

$$\frac{dv}{dT} = \frac{K_1}{2v}$$

If this equation is integrated between $v_1 v_2$ and $t_1 t_2$ and the values of v_1 and v_2 are chosen so that

$$\frac{v_2 + v_1}{2} = v$$

we get

$$v_2^2 - v_1^2 = K_1(t_2 - t_1) \quad (13)$$

which gives on rearrangement

$$\frac{v_2 - v_1}{t_2 - t_1} = \frac{K_1}{v_2 + v_1}$$

or

$$\frac{v_2 - v_1}{t_2 - t_1} = \frac{K_1}{2} \times \frac{1}{\frac{v_2 + v_1}{2}}$$

and

$$\frac{\Delta v}{\Delta t} = \frac{K_1}{2} \left(\frac{1}{\frac{v_2 + v_1}{2}} \right) \quad (14)$$

Since the condition that $\frac{v_2 + v_1}{2} = v$ was imposed originally, Equation (14) may be written

$$\frac{\Delta v}{\Delta t} = \frac{K_1}{2v} \quad (15)$$

which is exactly the same value that is obtained for dv/dt by differentiating Equation (12).

Hence by using $\Delta v/\Delta t$ and the value of $\pi PR^4/8 l$ for the point midway between v_1 and v_2 or which is the same l_1 and l_2 , the same result is obtained as when the instantaneous value of the rate of flow dv/dt is used.

These considerations are based on the assumption that R is constant. As no capillary is absolutely uniform the above proof is not absolutely rigid as the v/t curve will deviate slightly from the truly parabolic. However, the error incurred here is very slight and less than the experimental error of timing especially at the inlet end of the tube. By this method the necessity of correcting the observed time, in order to evaluate dv/dt is eliminated.

The following tables show the results of some experiments on castor oil. Tables I, II and III give the dimensions of the tubes and the values of $\pi PR^4/8 \left(\frac{l_2 + l_1}{2} \right)$ and $\left(\frac{PR}{2(l_2 - l_1)} \ln \frac{l_2}{l_1} \right) \frac{\pi R^3}{4}$. In these tables the headings have the following meanings:

l = distance from the end of the tube in centimeters.

R = average radius from the end of the tube to l .

Δv = the volume in cc. between successive graduations.

The meanings of the others are obvious from what has been said above.

Tables IV, V and VI give the results on three experiments with castor oil in the three tubes. In these tables

$$\phi_1 \text{ is } \frac{\Delta v}{\Delta t} / \frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)}$$

$$\phi_2 \text{ is } \frac{\Delta v}{\Delta t} / \frac{\pi PR^4}{8(l_2 - l_1)} \ln \frac{l_2}{l_1}$$

and

$$\phi_3 \text{ is } \frac{dv}{dt} / \frac{\pi PR^4}{8l}$$

TABLE I
CAPILLARY TUBE No. II

l	R	Δv	$\frac{\pi PR^4}{8 l}$ $\times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)}$ $\times 10^4$	$\frac{\pi R^4}{4} \left(\frac{PR}{2(l_2 - l_1)} \ln \frac{l_2}{l_1} \right)$ $\times 10^4$
5					
10	0.04823	0.0366	2.829	3.777	3.958
15	0.04823	0.0367	1.885	2.266	2.294
20	0.04816	0.0362	1.406	1.615	1.612
25	0.04809	0.0359	1.118	1.249	1.247
30	0.04800	0.0358	0.925	1.015	1.011
35	0.04800	0.0363	0.799	0.854	0.856
40	0.04807	0.0370	0.698	0.743	0.745
45	0.04818	0.0374	0.626	0.657	0.660
50	0.04830	0.0382	0.569	0.597	0.600
55	0.04841	0.0383	0.522	0.545	0.548
60	0.04849	0.0382	0.482	0.490	0.503
65	0.04857	0.0383	0.448	0.465	0.466
70	0.04862	0.0383	0.417	0.433	0.434
75	0.04864	0.0377	0.390	0.403	0.404

TABLE II
CAPILLARY TUBE No. IV

l	R	Δv	$\frac{\pi PR^4}{8 l}$ $\times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)}$ $\times 10^4$	$\frac{\pi R^4}{4} \left(\frac{PR}{2(l_2 - l_1)} \ln \frac{l_2}{l_1} \right)$ $\times 10^4$
5					
10	0.0504	0.0394	3.378	4.563	4.711
15	0.0503	0.0388	2.238	2.691	2.731
20	0.0501	0.0388	1.649	1.900	1.879
25	0.0500	0.0382	1.305	1.460	1.460
30	0.0499	0.0375	1.083	1.185	1.194
35	0.0497	0.0374	0.914	0.991	0.994
40	0.0496	0.0371	0.793	0.848	0.850
45	0.0495	0.0369	0.699	0.742	0.744
50	0.0494	0.0366	0.624	0.659	0.660
55	0.0493	0.0364	0.563	0.591	0.593
60	0.0492	0.0362	0.512	0.536	0.537
65	0.0491	0.0360	0.468	0.489	0.490
70	0.0490	0.0357	0.431	0.449	0.449
75	0.0489	0.0355	0.399	0.415	0.415

TABLE III
CAPILLARY TUBE No. VII

l	R	Δv	$\frac{\pi PR^4}{8 l}$ $\times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)}$ $\times 10^4$	$\frac{\pi R^4}{4} \left(\frac{PR}{2(l_2 - l_1)} \ln \frac{l_2}{l_1} \right)$ $\times 10^4$
5					
10	0.0693	0.0744	12.04	16.10	16.71
15	0.0692	0.0758	8.02	9.63	9.75
20	0.0693	0.0753	6.02	6.88	6.93
25	0.0693	0.0765	4.83	5.37	5.40
30	0.0694	0.0764	4.05	4.45	4.43
35	0.0695	0.0764	3.48	3.75	3.76
40	0.0695	0.0769	3.06	3.26	3.27
45	0.0696	0.0774	2.72	2.89	2.89
50	0.0697	0.0774	2.46	2.59	2.59
55	0.0697	0.0779	2.25	2.35	2.38
60	0.0698	0.0796	2.07	2.16	2.15
65	0.0699	0.0802	1.92	2.00	2.00
70	0.0701	0.0809	1.81	1.86	1.87
75	0.0702	0.0818	1.70	1.75	1.75

TABLE IV
CASTOR OIL
Experiment No. 233. Tube No. II. $P = 28.3$ mm. Hg.

l	t_r	T	$\frac{\Delta v}{\Delta t} \times 10^4$	$\frac{dv}{dt} \times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_0 + l_1}{2} \right)} \times 10^4$	$\frac{\pi PR^4}{8(l_0 - l_1)} \ln \frac{l_0}{l_1} \times 10^4$	$\frac{\pi PR^4}{8l} \times 10^4$	ϕ_1	ϕ_2	ϕ_3
5										
10	16.2	22.0	22.6	16.6	107.0	112.0	79.8	0.211	0.202	0.208
15	43.6	49.4	13.4	11.1	64.3	64.8	53.4	0.209	0.206	0.208
20	82.0	87.8	9.43	8.34	45.6	45.7	39.8	0.207	0.206	0.209
25	130.8	136.6	7.36	6.66	35.4	35.3	31.6	0.208	0.208	0.210
30	189.6	195.4	6.09	5.57	28.7	28.6	26.2	0.212	0.213	0.212
35	259.3	265.1	5.21	4.79	24.2	24.2	22.6	0.216	0.215	0.212
40	340.8	346.6	4.54	4.18	21.0	21.1	19.8	0.216	0.215	0.211
45	435.6	441.4	3.95	3.73	18.6	18.7	17.7	0.212	0.211	0.211
50	539.6	545.4	3.67	3.36	16.9	17.0	16.1	0.217	0.216	0.208
55	655.4	661.2	3.31	3.06	15.4	15.5	14.8	0.215	0.214	0.207
60	779.4	785.2	3.08	2.82	13.9	14.2	13.6	0.222	0.217	0.207
65	916.2	922.0	2.80	2.61	13.2	13.2	12.7	0.213	0.212	0.206
70	1061.4	1067.2	2.64	2.43	12.25	12.3	11.8	0.216	0.214	0.206
75	1214.6	1220.4	2.46	2.28	11.4	11.4	11.0	0.216	0.216	0.207
$t_0 = 5.8$										
Average ...								0.2135	0.2118	0.2087
Av. Dev.								0.0034	0.0037	0.0018
% Dev.								1.6	1.7	0.86

TABLE V
Castor Oil
Experiments Nos. 227 and 231. Tube No. IV. $P = 28.2$ mm. Hg.

l	t_r	T	$\frac{\Delta v}{\Delta t} \times 10^4$	$\frac{dv}{dt} \times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_0 + l_1}{2} \right)} \times 10^4$	$\frac{\pi PR^4}{8(l_0 - l_1)} \ln \frac{l_0}{l_1} \times 10^4$	$\frac{\pi PR^4}{8l} \times 10^4$	ϕ_1	ϕ_2	ϕ_3
5										
10	14.2	17.6	27.8	23.2	128.7	132.8	95.3	0.216	0.209	0.244
15	37.6	41.0	16.6	14.4	76.0	77.0	63.2	0.219	0.216	0.228
20	71.1	74.5	11.6	10.5	53.6	53.0	46.5	0.217	0.219	0.226
25	114.1	117.5	8.88	8.30	41.2	41.2	36.7	0.216	0.216	0.226
30	164.5	167.9	7.44	6.92	33.4	33.7	30.5	0.223	0.221	0.226
35	225.3	228.7	6.15	5.89	28.1	28.0	25.8	0.219	0.220	0.228
40	296.5	299.7	5.21	5.12	23.9	24.0	22.4	0.218	0.217	0.228
45	374.2	377.6	4.75	4.55	21.0	21.0	19.7	0.226	0.226	0.231
50	463.0	466.4	4.12	4.08	18.6	18.6	17.6	0.222	0.222	0.232
55	560.5	563.9	3.73	3.69	16.7	16.7	15.9	0.223	0.223	0.232
60	673.0	673.0	3.32	3.36	15.1	15.1	14.4	0.220	0.220	0.233
65	785.2	788.6	3.12	3.10	13.8	13.8	13.2	0.226	0.226	0.235
70	912.1	915.5	2.82	2.86	12.7	12.7	12.2	0.222	0.222	0.234
75	1047.3	1050.7	2.62	2.66	11.7	11.7	11.3	0.224	0.224	0.235
$t_0 = 3.40$							Average ...	0.221	0.220	0.232
							Av. Dev. ...	0.0031	0.0036	0.0035
							% Dev. ...	1.4	1.6	1.5

TABLE VI
CASTOR OIL
Experiments Nos. 209 and 211. Tube No. VII. $P = 27.6$ mm. Hg. 30° .

l	t_r	T	$\frac{\Delta v}{\Delta t} \times 10^4$	$\frac{dv}{dt} \times 10^4$	$\frac{\pi PR^4}{8 \left(\frac{l_2 + l_1}{2} \right)} \times 10^4$	$\frac{\pi PR^4}{8(l_2 - l_1)} \ln \frac{l_2}{l_1} \times 10^4$	$\frac{\pi PR^4}{8l} \times 10^4$	ϕ_1	ϕ_2	ϕ_3
5	7.6	10.2	97.9	73.6	445.0	462.0	332.0	0.220	0.212	0.222
10	20.2	22.8	60.1	49.6	266.0	269.0	221.0	0.226	0.227	0.224
15	37.5	40.1	43.5	37.5	190.0	191.0	166.0	0.229	0.228	0.226
20	60.0	62.6	34.0	30.2	148.0	149.0	133.0	0.230	0.228	0.226
25	87.5	90.1	27.8	25.2	123.0	122.0	111.8	0.226	0.228	0.226
30	120.3	122.9	23.3	21.6	104.0	104.0	96.2	0.224	0.224	0.225
35	157.7	160.3	20.5	18.8	90.0	90.2	84.5	0.228	0.227	0.223
40	199.5	202.1	18.5	17.0	79.8	79.8	75.2	0.232	0.232	0.226
45	247.1	249.7	16.3	15.3	71.5	71.5	68.0	0.228	0.228	0.225
50	300.2	302.8	14.7	13.9	64.9	64.6	62.0	0.226	0.228	0.224
55	358.8	361.4	13.5	12.7	59.6	59.4	57.2	0.226	0.227	0.222
60	422.2	424.8	12.6	11.8	55.2	55.2	53.0	0.228	0.228	0.223
65	492.0	494.6	11.6	10.9	51.4	51.5	50.0	0.226	0.226	0.218
70	565.8	568.4	11.1	10.2	48.3	48.3	46.9	0.230	0.230	0.218
75	$t_o = 2.6$									
							Average ...	0.2274	0.2266	0.2234
							Av. Dev. ...	0.0015	0.0025	0.0021
							% Dev. ...	0.65	1.15	0.94

In all cases the value of ϕ_2 as calculated by the Bingham-Murray method is low near the inlet end of the tube. There is no drift in the value of the fluidity as calculated by any of the methods. The agreement of the methods is fair and can probably be improved as can also the accuracy of the individual experiments. The lowest average deviation is 0.65% and the highest 1.7%. There is a 6% difference between the highest and lowest value by all methods of calculation and both of these extremes are by the method in which the correction was added to the observed time. The extremes of the other measurements are about 2.5% apart.

The results in these three tables seem to show a drift in the value of the fluidity with the radius but this is purely accidental as is shown by the results in Table VII which gives the fluidities in several tubes as calculated according to the equation for ϕ_2 .

$$\phi_2 = \frac{\Delta v}{\Delta t} \bigg/ \frac{\pi P R^4}{8 \left(\frac{l_2 + l_1}{2} \right)}$$

TABLE VII

FLUIDITY OF CASTOR OIL IN DIFFERENT TUBES

 R_1 = Average Radius from $l = 0$ to $l = 5$ cm. R_2 = " " " " $l = 0$ " $l = 75$ "

Tube	R_1	R_2	ϕ_2	Av. Dev.	Per Cent Dev.
II	0.0482	0.0486	0.2135	0.0034	1.6
IV	0.0504	0.0489	0.2211	0.0031	1.4
V	0.0576	0.0541	0.2242	0.0016	0.73
VII	0.0693	0.0702	0.2274	0.0015	0.65
VII	0.0693	0.0702	0.2250	0.0028	1.3
X	0.0306	0.0311	0.2121	0.0038	1.8
XI	0.0762	0.0758	0.2193	0.0044	2.0
XII	0.1095	0.1103	0.2215	0.0034	1.5
XII	0.1095	0.1103	0.2153	0.0035	1.6

Average = 0.2196

Conclusions

From the foregoing it is evident that the formula proposed by Bingham and Murray for calculating the shearing stress is not the correct one to use in conjunction with $\Delta v/\Delta t$ as the rate of flow. It has been shown that the rate of flow calculated as $\Delta v/\Delta t$ should be used with the actual value of $\pi P R^4/8l$ at a point midway between v_1 and v_2 to get the correct value of the fluidity. This method when properly used will give values of fluidity within about 1%.

The results of experiments on the flow of rubber sols in this instrument will be published in the near future.

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Research Laboratories Goodyear Tire and Rubber Company,
Akron, Ohio.

A MOTION PICTURE STUDY OF THE INFLUENCE OF GELATIN ON RATES OF CRYSTAL GROWTH AND SOLUTION OF COPPER SULFATE

BY WESLEY G. FRANCE

One of the first investigations of colloidal phenomena in which the motion picture camera was used, was made by Victor Henri,¹ who photographed the Brownian Movement of rubber latex particles using a high powered microscope.

The motion picture camera was not, however, combined with the ultramicroscope until two or three years later. At this time Jean Comandon² succeeded in photographing ultramicroscopically minute organisms and particles of colloidal dimensions showing marked Brownian Movement.

The first work of a similar nature conducted in our laboratory was a motion picture investigation of the Brownian Movement of Basic Lead Carbonate suspensions and Red Gold sols both in the presence and absence of gelatin. A preliminary report of this investigation was prepared for the April, 1924, meeting of the American Chemical Society and the abstract was published, with the abstracts of the Inorganic and Physical Chemistry division.³

Due to the inability of the writer to attend the meeting this paper was presented by title only.

Among the previous investigations of especial interest to colloid chemists are those of Darke, McBain and Salmon⁴ on soap structures and W. G. and H. C. Eddy⁵ on emulsions, and subsequently the work of E. O. Kraemer,⁶ E. P. Wightman and A. P. H. Trivelli, "Motion Picture Study of Rubber Latex Particles,"⁷ and a similar study by W. J. Kelly presented at the 1925 spring meeting of the American Chemical Society.

The fact that the motion picture camera has not met with more general use in colloidal investigations can be attributed to the following

¹ *Société Française de Physique—Bulletin des Séances*, 4, 45, 61 (1908).

² F. A. Talbot, "Practical Cinematography and its Applications," p. 169, J. B. Lippincott, 1913.

³ Abstracts of Papers to be presented before the Division of Inorganic and Physical Chemistry at the Washington meeting, No. 80.

⁴ *Proc. Roy. Soc. (London)*, 98A, 895 (1921).

⁵ *J. Ind. Eng. Chem.*, 13, 1016 (1921).

⁶ "Studies with the Kinoultramicroscope," Colloid Symposium Monograph, Vol. 11, p. 57.

⁷ *J. Ind. Eng. Chem.*, 17, 164 (1925).

two reasons: one, the considerable expense entailed in the purchase of standard motion picture equipment and the other, the difficulty and inconvenience encountered in the development of standard 35 mm. film in the ordinary dark room. These difficulties have been largely overcome by the advent of the 16 mm. film and moderately priced and readily procurable equipment for its use.

The present paper has for its object the demonstration of the use of this small film in connection with a preliminary study of the effect



FIG. 1.—Arrangement of apparatus for photographing microscopic crystals.

of gelatin on the rates of growth and rates of solution of copper sulfate crystals. One of the earliest investigations on the growth of copper sulfate crystals in which a photographic record was made is that of T. W. Richards and E. H. Archibald.⁸

Time will not permit outlining and discussing the large number of investigations on crystallization and solution velocities conducted by such investigators as J. H. Walton and co-workers, Rene Marcelin, M. Volmer, P. Niggli, R. Marc, J. J. P. Valetton, M. Kimura and others. Attention is directed, however, to the fact that in many cases these inves-

⁸ *Am. Chem. Jr.*, 28, 61-74 (1901).

tigations are concerned with the rates at which crystallization progresses through a definite volume of a saturated solution, whereas the present work is concerned solely with the rates of growth and solution of individual crystals of both microscopic and macroscopic dimensions.

Figure 1 shows the arrangement of the apparatus used for photographing crystals of microscopic dimensions. It consists essentially of a suitable support holding a Bell and Howell Filmo Camera vertically above a microscope provided with a binocular eyepiece. One ocular of the eyepiece is focused on a ground glass, which is in the same relative position as the film in the camera, on which the other ocular is focused. This enables one to follow visually the growth of the crystals and at the same time provides the necessary means for maintaining the crystal accurately focused on the film.



FIG. 2.—Arrangement of apparatus for photographing microscopic crystals.

Figure 2 shows the arrangement of the apparatus when used for photographing crystals of macroscopic dimensions. This is practically the same as in Figure 1 except that a 4" Dallmeyer Telephoto lens fitted with a binocular eyepiece is substituted for the microscope and illumination is provided by means of a microscope lamp placed below a glass plate supporting the vessel in which the crystal is growing or dissolving.

The procedure followed in photographing the microscopic crystals was as follows: A drop of copper sulfate solution saturated at 45°

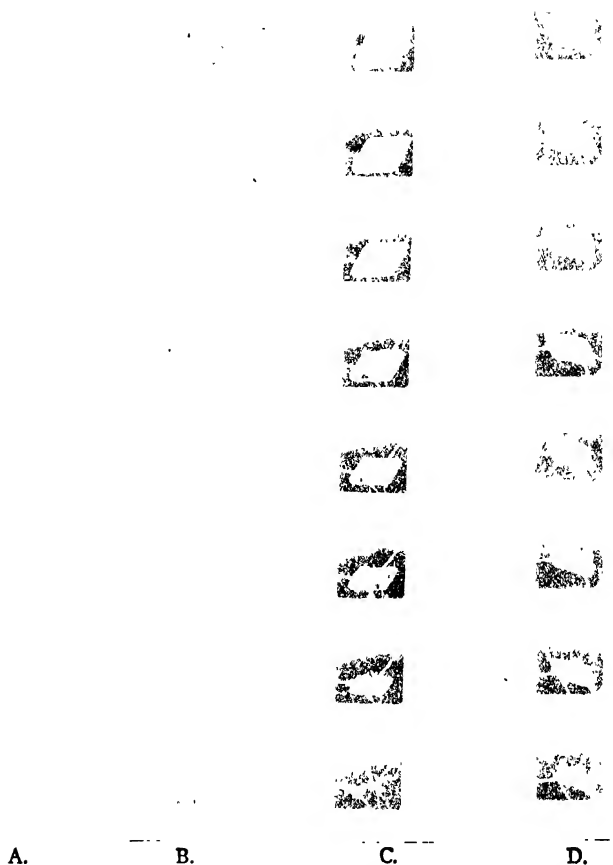


FIG. 3.

A. Microscopic copper sulfate crystal growing from solution containing no gelatin. Magnification 10 X.

B. Microscopic copper sulfate crystal growing from solution containing 0.5% gelatin. Magnification 10 X.

C. Macroscopic copper sulfate crystal dissolving in distilled water. Actual size as shown.

D. Macroscopic copper sulfate crystal dissolving in distilled water containing 3.0% gelatin. Actual size as shown.

was placed on a microscope slide, previously heated to this same temperature, the slide was then placed on the microscope stage and allowed to cool to 30°, and photographs were made at definite time intervals

during crystallization. In the case of the macroscopic crystals, a crystal about 3 mm. in length was placed in the solution saturated at 45° and while being cooled to 30° exposures were made of the growing crystal at definite time intervals. To determine the effect of gelatin on the rates of crystal growth the same procedure was followed except that the solutions in which the crystals were growing contained definite concentrations of gelatin. For the measurement of the rates of solutions, macroscopic crystals only were used. These were weighed and placed

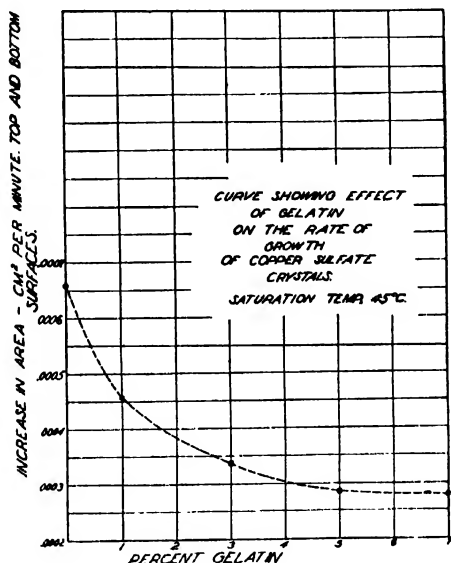


FIG. 4.

on a thin platinum wire support in a small vessel containing distilled water to which had been added definite concentrations of gelatin. Successive photographs were made at definite time intervals during solution.

Fig. 3-A shows a microscopic crystal of copper sulfate growing from a saturated solution at 45° containing no gelatin. The time interval between successive exposures is one minute. Figure 3-B is the photographic record of a microscopic copper sulfate crystal growing in a saturated solution at 45° to which 0.5% gelatin has been added. The time interval is one minute. Figure 3-C shows a macroscopic copper sulfate crystal going into solution in distilled water, and Fig. 3-D is the photographic record of a macroscopic copper sulfate crystal going

into solution in distilled water to which 3% gelatin has been added. In each case the time interval is one minute.

To determine the rates of growth and rates of solution, the negatives obtained as above described were projected on a calibrated screen and the area of the projected image measured directly. From the values so obtained for each successive exposure representing a known time interval the change in area, either an increase or decrease, per minute was calculated. Since this method of measurement does not include the

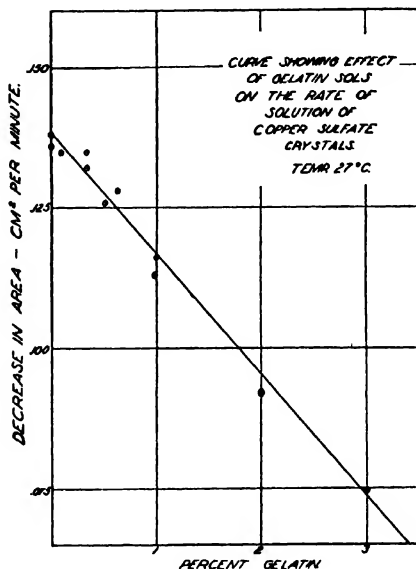


FIG. 5.

change in thickness of the crystals the results obtained can only be considered as semi-quantitative and are subject to revision when a method is used in which the thickness of the crystal is either directly measured or calculated from its weight, density and projected area, and from which the total area can be determined.

The curves in Figures 4 and 5 show the effect of gelatin on the rates of growth and rates of solution of copper sulfate crystals.⁹

The areas expressed in Fig. 4 include only the top and bottom planes of the growing crystals, whereas in Fig. 5 the areas are expressed for the

⁹ The data used for the construction of these curves were obtained by Mr. T. S. Eckert of this department.

entire crystal surfaces. The following conclusions, subject to the limitations imposed by the method of calculating the areas, can be drawn from these preliminary measurements.

1. The use of the 16 mm. motion picture camera for obtaining a photographic record of both growing and dissolving crystals is thoroughly satisfactory.

2. In the case of copper sulfate the rate of solution of individual crystals in pure water is very much greater than the rate of growth of individual crystals from saturated solutions.

3. The presence of small concentrations of gelatin affects the rate of growth to a greater extent than it affects the rate of solution of copper sulfate crystals.

In order that more accurate measurements may be made apparatus is at present being constructed which will give the exact weight of the crystals coincidently with the photographing of the top and side views.

The Ohio State University,
Columbus, Ohio.

